## REMARKS

Claims 7 and 38-54 are currently pending in the application, and are presented for further examination. Applicants respond below to the specific rejections raised by the Examiner in the Office Action mailed August 30, 2005.

**Rejections Under 35 U.S.C. § 102(b) - Rath reference**

The Examiner has rejected Claims 7, 38, 39, 41, 42, 44-49 and 53 under 35 U.S.C. § 102(b) as being anticipated by Rath (U.S. Patent No. 6,693,129, hereinafter "Rath"). According to the Examiner, Rath discloses a method of treating high LDL and high triglycerides by administering a composition that contains biotin and chromium (as chromium glycinate) in amounts falling within the scope of Applicants' claims. The Examiner asserts that Applicants have not provided evidence that the transition phrase "consisting essentially of" in Claim 7 excludes the additional compounds present in the Rath composition. The Examiner also asserts that "the composition for the treatment indicated [in Rath]" will inherently raise serum HDL cholesterol levels. For the reasons set forth below, Applicants respectfully disagree.

*The Rath Composition Includes Compounds Excluded by the phrase "Consisting Essentially of" in Claim 7*

Under 35 U.S.C. § 102(b), a claim is anticipated only if the reference reads on the claim. Claim 7 of the present application recites "A method for treating dyslipidemia *consisting essentially of* administering to an individual in need thereof a synergistically effective dose of chromium and biotin. . ." For the reasons set forth below, Rath does not read on Claim 7.

As set forth in Section 2111.03 of the M.P.E.P. and acknowledged by the Examiner, the transitional phrase "consisting essentially of" has a well-established meaning. As correctly noted by the Examiner, the transitional phrase "consisting essentially of" occupies a middle ground between claims that recite the transitional phrase "consisting of," which exclude all materials other than those recited in the claim, and claims that recite the transitional phrase "comprising," which do not exclude additional, unrecited elements. The transitional phrase "consisting essentially of" limits the scope of a claim by excluding additional materials or steps that materially affect the basic and novel characteristics of the invention. *Office Action* at 3-4; *Atlas Pander Co. v. E.I. DuPont de Nemours & Co.*, 750 F.2d 1569, 224 (Fed. Cir. 1998).

Federal Circuit law dictates that any ingredient that materially alters the basic and novel characteristics, regardless of the nature of the effect, *i.e.,* whether it enhances or otherwise alters those characteristics, is excluded by claims using the phrase "consisting essentially of." *See, e.g., PPG Industries v. Guardian Industries Corp.*, 156 F.3d 1351, 1357(Fed. Cir. 1998) (holding that materials not listed in claims to glass that cause measurable changes in the properties of the glass must be excluded where the claim uses "consisting essentially of" but not making a distinction between complementary or deleterious effects on the glass); *See also, Prody and Carberry Corp. v. Land O' Lakes, Inc.*, 97 F.Appx. 921, 928 (Fed. Cir. 2004) (holding that claims to a coffee lightener that include the phrase "consisting essentially of" would exclude ingredients that enhanced whitening properties of the lightener); *American Machine& Foundry Co. v. Ligget & Meyers Tobacco Co.*, 172 F.Supp 12 (D.C.N.J. 1959) (holding that the transition phrase "consisting essentially of" excluded materials that had a complementary or beneficial effect on tobacco paper).

Applicants note the Examiner's assertion that "absent a clear indication in the specification of what the basic and novel characteristics actually are, "consisting essentially of" will be construed as equivalent to comprising." *Office Action* at 4. Applicants maintain that in the instant case, the basic and novel characteristics of Claim 7, *e.g.* a method of treating dyslipidemia and synergistically raising serum HDL cholesterol levels, is readily apparent from the claims and the specification. Specifically, paragraph [0006] explains that symptoms of dyslipidemia include elevation of the serum total cholesterol, LDL cholesterol and triglyceride concentrations, and a decrease in HDL concentration. The synergistic effect of chromium and biotin on serum HDL levels is illustrated in Figure 14. Accordingly, any compound that has a positive or deleterious affect on serum levels of total cholesterol, LDL cholesterol, triglyceride, or HDL cholesterol materially affects the basic and novel characteristics of Applicants' claimed invention.

Rath discloses a composition and method for lowering plasma Lp(a) levels in humans. The Rath composition is disclosed in Table 1, beginning on Column 6, line 45 and includes no less than thirty-five compounds in addition to biotin and chromium, including: ascorbic acid, arginine, L-carnitine, folic acid, niacin, and others.

Claim 7 relates to a method of treating dyslipidemia consisting essentially of administering a synergistically effective dose of a chromium/biotin complex to an individual. On the other hand, the Rath composition includes ascorbic acid, niacin, L-carnitine, and numerous other compounds. Applicants provide evidence herewith demonstrating that the additional compounds in the Rath composition are bioactive, and therefore the Rath composition fails to meet the limitation of a method for treating dyslipidemia consisting essentially of administering chromium and biotin to an individual in need thereof. Exhibit A, a publication by the Allergy Research Group®, reports that niacin and L-carnitine have been shown to optimize LDL and HDL cholesterol levels. Exhibit B, an informational publication about ascorbic acid (vitamin C), states that several studies suggest that vitamin C helps decrease total and LDL cholesterol and triglycerides, as well as increase HDL levels. Applicants note that ascorbic palmitate, another compound in the Rath composition is merely a fat soluble form of vitamin C. As discussed above, vitamin C is known to be effective in decreasing total and LDL cholesterol levels and triglyceride levels. Exhibit C, (previously submitted) reports that L-arginine reduces hyperlipidemia. Exhibit D (previously submitted) demonstrates that folic acid reduces LDL levels. In other words, the Rath composition clearly contains several bioactive ingredients that materially affect serum cholesterol and triglyceride levels, thereby materially affecting the novel and basic characteristics of Applicants' claimed invention.

Applicants note that in the previous Office Action mailed on February 24, 2005, the Examiner reasoned that "addition of folic acid, L-arginine, or L-carnitine would not materially affect the basic and novel characteristics of the invention as they provide similar benefits desired by Applicant in the claimed invention." Office Action at p. 6. As stated above, the law does not distinguish between additional compounds that enhance or have a deleterious effect on a claimed composition in determining whether a compound is properly excluded by the transitional phrase "consisting essentially of." Applicants' ample objective evidence demonstrating that several compounds present in the Rath composition materially affect the basic and novel characteristics of Applicants' claimed invention illustrate that the Rath composition fails to meet the limitation of administering to an individual a composition "consisting essentially of" chromium and biotin. Accordingly, the Rath composition falls outside of the scope of Applicants' claims and cannot anticipate Claim 7 under 35 U.S.C. § 102(b).

*Rath does not expressly or inherently disclose each and every limitation in Claims 38, 39, 41, 42, 44-49 and 53*

Claims 38-54 recite "[a] method for raising serum HDL levels, comprising administering to an individual in need thereof a synergistically effective dose of chromium complex and biotin." Accordingly, Applicants' claims require the step of identifying an individual in need of raising serum HDL levels. Rath is completely silent regarding serum HDL levels. Therefore, Rath fails to meet the limitation of identification of an individual in need of raising serum HDL levels. The Examiner argues, however, that "the group of individuals which would benefit from raised serum HDL cholesterol levels. . .are not patentably distinguishable from the. . .population of individuals disclosed in the prior art." Office Action at 2. Applicants disagree.

The Rath reference fails to expressly teach the limitation of "administering to an individual in need" of a composition to raise serum HDL levels. Therefore, the Examiner appears to be relying on the doctrine of inherency to establish that Rath is anticipatory. "A claim limitation is inherent in the prior art if it is necessarily present in the prior art, not merely probably or possibly present." *Akamai Technologies, Inc. v. Cable & Wireless Internet Svcs., Inc.* 344 F.3d 1186 (Fed. Cir. 2003). Further, in relying upon the theory of inherency, the Examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. *Ex parte Levy,* 17 U.S.P.Q.2d 1461, 1464 (B.P.A.I. 1990). Accordingly, in order to establish that Rath is anticipatory, the Examiner must establish that individuals in Rath would necessarily - not probably or possibly - be in need of a composition that raises serum HDL levels. As discussed above, Rath discloses a composition that reduces serum Lp(a) levels, total cholesterol levels, LDL and triglyceride levels. However, while individuals in need of the composition in Rath may possibly also be in need of a composition that raises serum HDL levels, this is not necessarily the case. In fact, several studies have identified Lp(a) levels, triglyceride levels, and HDL levels as independent risk factors for disorders such as coronary heart disease (CHD) and stroke. In a study that examined the isolated effects of high triglycerides on stroke, the researchers "tease[d] out a number of other risk factors. . .including low HDL cholesterol." The researchers found that high triglycerides were a risk factor for stroke independent of the other risk factors. LaRusso, L., (2005), "High blood triglycerides identified as independent risk

factor for stroke," attached as Exhibit E. Assmann, et al. reached a similar conclusion when analyzing the effects of elevated triglycerides on CHD. Assmann, et al. (1998), Eur. Heart J., Suppl. M:M8-14, attached as Exhibit F. The Assmann et al. study concluded that fasting levels of triglycerides were an independent risk factor for CHD events, irrespective of serum levels of HDL. Notably, the authors also found that other independent predictors of CHD include LDL and HDL. Accordingly, Applicants submit that individuals identified in Rath as in need of therapeutic intervention would not necessarily be in need of therapeutics to raise serum HDL levels. Likewise, Applicants submit that there are populations of individuals in need of raising serum HDL levels who do not necessarily need a therapeutic to lower serum LDL levels or triglycerides. By way of example, some individuals suffer from a condition referred to as hypoalphalipoproteinemia, which is characterized by low HDL cholesterol levels without elevated triglycerides or LDL levels. See, Ginsburg, et al. (1991), Am J. Cardiol. 68:187-192, attached as Exhibit G. In view of the above, Applicants respectfully submit that the Examiner has failed to provide evidence to support the determination that "the group of individuals which would benefit from raised serum HDL cholesterol levels. . .are not patentably distinguishable from the. . .population of individuals disclosed in the prior art." Applicants' evidence establishes that the groups are patentably distinct. The above evidence demonstrates that Rath fails to teach each and every limitation of the rejected claims, either expressly or inherently, which include the step of "administering to an individual in need [of a composition to elevate serum HDL levels]." Therefore, Rath is not anticipatory under 35 U.S.C. § 102(b).

For the reasons set forth above, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 102(b).

**Rejections under 35 U.S.C. § 103(a) - Rath reference**

Although not discussed separately from the rejection under 35 U.S.C. § 102(b), the Examiner also rejected Claims 7, 38, 39, 41, 42, 44-49 and 53 under 35 U.S.C. § 103(a) as being obvious in view of Rath. In the previous Office Action, the Examiner maintained that "the Graham v. John Deere factors are not applicable in an inherency-based rejection." Office Action dated February 24, 2005, p. 6. Applicants have not found guidance either in the C.F.R. or the M.P.E.P. that indicates that the Graham v. John Deere factors are ever not applicable in a

rejection under 35 U.S.C. § 103(a), and respectfully request that the Examiner provide Applicants with the relevant rule.

The law dictates that in order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the teachings of the references. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all the claim limitations. M.P.E.P. §2143. As discussed below, the differences between Applicants' invention and the Rath disclosure are significant and the reference is not sufficient to support a *prima facie* case of obviousness.

Regarding Claim 7, the Examiner has pointed to no motivation to modify the Rath patent to arrive at the claimed method of treating dyslipidemia or raising serum HDL cholesterol levels. The Rath composition includes over thirty-five ingredients. Nowhere does the Rath reference suggest the desirability or advantages of selecting only two of the almost forty components to treat dyslipidemia or raise serum HDL cholesterol levels as is presently claimed. Applicants submit that absent impermissible hindsight, it would not have been obvious to select only two bioactive components from the nearly forty components in the Rath composition to arrive at the method of treating dyslipidemia consisting essentially of administering a chromium complex and biotin. The Examiner previously conceded that "Examiner has never opined that it would have been obvious to omit the other thirty five ingredients." Nevertheless, the Examiner maintained that "there is no need to omit the thirty-five other bioactive ingredients as Applicant[s] ha[ve] not show[n] that they are excluded by the phrase 'consisting essentially of.'" Office Action mailed February 24, 2005, p. 6-7. Applicants respectfully submit that the Examiner is overlooking the requirement that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference. The Examiner has failed to point to any such evidence. Furthermore, as Applicants previously noted, there is great benefit in being able to narrow down the number of active ingredients one must use.

Additionally, regarding Claim 7, Applicants submit that the Examiner has not established that the Rath reference meets the second prong of the test for obviousness. Specifically, the Rath

reference teaches that a composition comprising several bioactive ingredients has an overall effect of lowering Lp(a), total cholesterol, LDL cholesterol and triglyceride levels. The Rath provides no teaching or suggestion with respect to individual compounds or any combination thereof. Applicants assert that, given the disclosure in the Rath reference, the skilled artisan would not have reason to suspect that from among the several compounds of the Rath combination, the combination of chromium and biotin alone would yield the unexpected synergistic results in raising serum HDL levels taught by Applicants.

Finally, as Applicants discuss in reference to the rejection under 35 U.S.C. § 102(b), Rath simply fails to teach or suggest all of the claim limitations in Claim 7, or in Claims 38, 39, 41, 42, 44-49 and 53. As discussed above, Claim 7 recites a method of treating dyslipidemia or raising serum HDL levels consisting essentially of administering a chromium complex and biotin. As noted above, Claim 7 excludes compounds such as niacin, Vitamin C, and others that are present in the Rath composition. This limitation is nowhere taught or suggested in Rath. As such, Rath cannot support a *prima facie* case of obviousness for Claim 7. Regarding Claims 38, 39, 41, 42, 44-49 and 53, Applicants have demonstrated above that the Rath reference fails to expressly or inherently teach the step of "administering a composition to an individual in need [of a composition to raise serum HDL levels]," as recited in the Claims. Thus, Rath also fails to support a *prima facie* case of obviousness for Claims 38, 39, 41, 42, 44-49 and 53.

In view of the above, Applicants respectfully submit that the rejection under 35 U.S.C. § 103(a) is improper, and request that the Examiner reconsider and withdraw the rejection.

**Rejection Under 35 U.S.C. § 102(b) - McCarty Reference**

The Examiner has maintained the rejection of Claims 7, 38-50, 53, and 54 under 35 U.S.C. § 102(b) as anticipated by McCarty et al. (U.S. Patent 5,929,066, "McCarty"). In particular, the Examiner alleges that McCarty discloses a method for reducing hyperglycemia and stabilizing the level of serum glucose by administering a synergistically effective amount of chromium complex and biotin falling within the scope of the present claims The Examiner points to U.S. Patent 6,140,304 to Sears ("Sears") as evidence that "it is inherent that reducing hyperglycemia and stabilizing the level of serum glucose will treat dyslipidemia and increase HDL cholesterol levels." Office Action at 5. Applicants respectfully disagree.

As discussed at length above, inherent anticipation requires that the missing descriptive material is 'necessarily present,' not merely probably or possibly present. *Trintec*, 295 F.3d at 1292. In relying upon the theory of inherency, the Examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d at 1464. Accordingly, in order to establish that McCarty inherently anticipates Claims 7, 38-50, 53, and 54, the Examiner has the burden of showing that treating dyslipidemia and increasing HDL cholesterol levels will necessarily flow from reducing hyperglycemia and stabilizing serum glucose levels. Applicants submit that the Examiner has not met this burden. The Examiner relies upon the teachings of Sears for this teaching. However, Sears merely states that "hyperinsulemia is associated with increased triglycerides, decreased HDL cholesterol levels, and elevated percent body fat." Thus, the Examiner is relying on the mere association of the condition of hyperinsulemia and altered lipid profiles, as proof that treating hyperglycemia would necessarily treat dyslipidemia and increase HDL cholesterol levels. Applicants assert that the mere association of the two conditions does not support the conclusion that "it is inherent that reducing hyperglycemia and stabilizing the level of serum glucose will treat dyslipidemia and increase HDL cholesterol levels." Office Action at 5. As discussed above with respect to the risk factors for CHD, the mere coincidence of symptoms does not establish a causal connection between them.

Applicants submit that some cases of hyperglycemia arise as a result of hyperinsulemia or insulin resistance, but that other causes of hyperglycemia also exist. Furthermore, in the cases where hyperglycemia arises from a cause other than insulin resistance, for example in the case of Type I Diabetes, hyperglycemia is not associated with elevated levels of triglycerides and decreased HDL levels. See, Roberts, R., (2003), "What Type of Diabetes Do I Have?" Attached as Exhibit H. Furthermore, in instances where dyslipidemia arises from causes completely unrelated to hyperglycemia, a method useful for the treatment of hyperglycemia would not necessarily alleviate dyslipidemia or increase serum HDL levels. Applicants attach herewith Exhibit I, an abstract of journal article entitled "Drug-related dyslipidemia after renal transplantation" as proof of this concept. As the title suggests, dyslipidemia does not always arise, and is not always associated with, hyperglycemia. Thus, in cases where dyslipidemia arises

as a result of renal transplantation, reducing serum glucose levels will not necessarily treat dyslipidemia. In view of the above evidence, Applicants submit that McCarty fails to teach each and every limitation of the rejected claims. In particular, McCarty, which relates to treating hyperglycemia, fails to disclose, either expressly or inherently, methods that will necessarily treat dyslipidemia or raise serum HDL levels.

Applicants have shown that McCarty fails to teach each and every limitation of Applicants' claims and therefore request that the Examiner withdraw the rejection under 35 U.S.C. § 102(b).

**Rejection Under 35 U.S.C. § 103(a) - McCarty Reference**

The Examiner maintains that Claims 7, 38-50, 53, and 54 are rendered obvious over McCarty, "because the prior art discloses products and uses that contain the same exact ingredients/compositions as that of the claimed invention." Office Action at 5. In support of his argument, the Examiner cites to *In re May* 197 USPQ 601, 607 (CCPA 1978) and *Ex parte Novitski* 26 U.S.P.Q.2d 1389, 1390-91 (BPAI 1993). Applicants respectfully disagree.

It is well-established that therapeutic uses of compounds disclosed in the prior art are patentable, provided the reference does not suggest the claimed therapeutic use. *Merck & Co. v. Teva Pharmaceuticals USA, Inc.*, 347 F.3d 1367 (Fed. Cir. 2003). As the Examiner correctly points out "McCarty et al.. . .discloses a method for reducing hyperglycemia and stabilizing the levels of serum glucose." Office Action at 5. As discussed at length above, the McCarty reference does not expressly or inherently disclose compositions or methods for treating dyslipidemia and raising serum HDL levels, the subject of the instant invention. Applicants reiterate their position that a teaching of a method for reducing hyperglycemia does not inherently teach a method for treating dyslipidemia, merely because there is an association between hyperinsulemia and dyslipidemia. Under *Merck,* therefore, because Applicants claims relate to novel therapeutic uses of biotin and chromium complexes, they are patentable over the McCarty reference which discloses a similar composition, but which fails to teach or suggest Applicants' presently claimed therapeutic use, *i.e.,* treating dyslipidemia and/or raising serum HDL levels.

The cases cited by the Examiner in support the rejection under 35 U.S.C. § 103(a) do not support the Examiner's assertion that the claims are unpatentably obvious over McCarty. The

claims at issue in *In re May* related to methods of using a particular stereoisomer of a compound to effect analgesic activity while exhibiting decreased dependency properties. The prior art disclosed that the racemate and various other isomers of the same compound had analgesic activity. Nevertheless, the Court determined that Applicants' claims were non-obvious in view of the prior art, finding that none of the references suggested that the claimed compound exhibited the claimed properties. *In re May,* 574 F.2d at 609. As discussed above, Applicants assert that the claims in the cited art and the present claims relate to two different therapeutic uses, *i.e.,* the treatment of hyperglycemia and/or stabilizing blood glucose levels, and dyslipidemia. Nevertheless, Applicants assert that, as was the case in *In re May*, nothing in the cited art (McCarty) suggests that a combination of chromium and biotin is useful for treating dyslipidemia or raising serum HDL levels. Thus, even if *In re May* were relevant to the instant case, the holding in *In re May* further supports Applicants' position that the claims are non-obvious over McCarty.

*In re Novitski* is equally insufficient to support the Examiner's position that the claims are obvious over McCarty. The disputed claim in *In re Novitski* related to a method of protecting a plant from plant pathogenic nematodes by inoculation with a particular bacterial species. The prior art disclosed a method that included the steps of inoculating a plant with a particular bacterial strain of the applicants' claimed species. The prior art, however, did not expressly disclose that the bacterial strain possessed nematode-inhibiting properties, nor did it expressly disclose a method for protecting a plant from nematodes. The court held that the claims were non-obvious, but inherently anticipated by the prior art reference because the method disclosed in the prior art "inherently and necessarily constitute[d] a method for protecting a plant from plant pathogenic nematodes." *In re Novitski* 26 USPQ at 1390-1391. (Emphasis added). In the instant case, McCarty discloses a method of treating hyperglycemia and/or stabilizing serum glucose levels. As discussed at length above, this method does not "inherently and necessarily" constitute a method for raising serum HDL levels or treating dyslipidemia. The cited references upon which the Examiner relies for the proposition that the claimed methods are inherently taught in the art merely teach an association between hyperinsulemia and hypercholesterolemia, and are insufficient to establish that treating hyperglycemia and stabilizing serum glucose levels

inherently treats dyslipidemia. As such, Applicants submit that *In re Novitski* does not support the Examiner's rejection under 35 U.S.C. § 103(a).

Furthermore, Applicants submit that the Examiner has failed to establish that the rejected claims are *prima facie* obvious over McCarty. First, the Examiner has failed to establish that the cited reference discloses each and every limitation of the claimed invention. As discussed fully in reference to the rejection under 35 U.S.C. § 102(b), McCarty fails to expressly or inherently teach or suggest each and every limitation of Claims 7, 38-50, 53, and 54. In particular, McCarty teaches a method for reducing hyperglycemia. McCarty does not expressly or inherently teach a method of treating dyslipidemia or a method of synergistically raise serum HDL levels. This alone demonstrates that the claimed invention is not obvious over McCarty. Nevertheless, Applicants also submit that the Examiner has failed to establish that the skilled artisan would have a reasonable expectation of success in treating dyslipidemia and raising serum HDL levels, given the disclosure in McCarty. As demonstrated above, hyperglycemia and dyslipidemia are two independent, often unrelated disorders. As such, given the disclosure that chromium and biotin is useful for treating hyperglycemia, and nothing more, the skilled artisan would not have a reasonable expectation that the same composition would be necessarily and always be useful in treating dyslipidemia or raising serum HDL levels. In view of the above, Applicants submit that the Examiner has failed to meet the three-prong test for obviousness set forth in M.P.E.P. §2143. Applicants therefore request that the Examiner withdraw the rejection under 35 U.S.C. § 103(a) over McCarty.

**Rejection Under 35 U.S.C. § 103(a) - McCarty, de la Harpe, Brand Miller, Rath and Sears References**

The Examiner maintains the rejection of Claims 7 and 38-54 as allegedly being unpatentably obvious over U.S. Patent No. 5,789,401 to McCarty ("the '401patent) or U.S. Patent 5,929,066 ("McCarty" ) each in view of U.S. Patent 5,948,772 to de la Harpe et al. ("de la Harpe") and Brand Miller (Am J. Clin. Nutr. (1994))("Brand Miller") as presented in previous Office Actions. The prior art references have been extensively characterized by both the Examiner and Applicants in previous communications. The rejection of the claims is maintained in the present Office Action, in view of the same prior art and in further view of Rath and Sears. According to the Examiner, Sears discloses that insulin resistance due to hyperinsulemia is

commonly associated with increased triglycerides, decreased HDL levels and elevated percent body fat. The Examiner argues that de la Harpe discloses that hypercholesterolemia is present in diabetics. The Examiner then declares that the skilled artisan would expect that biotin, known to be effective in controlling diabetes, would be effective in alleviating hypercholesterolemia. Applicants respectfully disagree, and note that the Examiner has not asserted that the skilled artisan would expect that biotin would be effective in raising serum HDL levels. Finally, the Examiner states that the Applicants' evidence of synergistic effects is not commensurate in scope with the breadth of the claims.

The Examiner's rejection over the cited references is based on the exact same assumptions as those presented in the 35 U.S.C. § 103(a) rejection over McCarty, i.e., "that one skilled in the art would expect that treating the underlying cause, i.e., diabetes, would be effective in treating the symptom, i.e., hypercholesterolemia." Office Action at 7. Emphasis added. The Examiner's reliance on Sears in support of the causal relationship is discussed above. The Examiner argues that "de la Harpe discloses that hypercholesterolemia is present in diabetics. ... Diabetics suffer from ineffective insulin and compromised glucose metabolism which leads to hypercholesterolemia." *Id.* Applicants assert that the teachings of de la Harpe - alone or in combination with the other cited references - are insufficient to establish that the combination of chromium and biotin (not mentioned in de la Harpe), would synergistically raise serum HDL levels. Simply, none of the cited references provide a causal link between hyperglycemia and lowered HDL levels.

Applicants previously asserted that the effectiveness of biotin in altering serum lipid levels is found exclusively in Applicants' specification. The Examiner disagrees, arguing that "the effectiveness of biotin in altering serum lipid levels in [*sic*] not found exclusively in Applicant's specification." Office Action at 8. However, the Examiner fails to expressly point to a reference that teaches that biotin - either alone or in combination with chromium - raises serum HDL levels. Applicants assume that the Examiner is relying on Rath for this teaching. However, as discussed at length, the Rath composition contains several bioactive ingredients known to affect serum lipid levels. One cannot draw the conclusion from these teachings that two of the over thirty-five compounds synergistically affects serum lipid levels. This line of reasoning is even more tenuous, given that Rath is completely silent about serum HDL levels.

As there is no suggestion that biotin supplementation would affect lipid levels or HDL levels in any way in the cited prior art, the use of biotin in combination with chromium, to affect a synergistic elevation in serum HDL levels is not obvious.

In view of the above, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness, and request that the Examiner withdraw the rejection under 35 U.S.C. § 103(a).

**Applicants have rebutted any *prima facie* case of obviousness with unexpected results**

For the reasons set forth above, Applicants have previously asserted that the synergistic effects of chromium and biotin on HDL levels is unexpected. The Examiner maintains, however, that the McCarty references disclose the combination of biotin and chromium complex results in synergistic effects, and concludes that "it is expected from the prior art that the combination of chromium complex and biotin would result in increased HDL cholesterol levels." Office Action at 9. The Examiner also argues that only specific amounts of chromium and biotin are tested, and that the evidence of synergy is not commensurate in scope with the breadth of the claims. Applicants disagree.

As discussed above, the teachings of the McCarty references are limited to methods of reducing hyperglycemia and stabilizing serum glucose levels. The Examiner correctly asserts that the McCarty references disclose a synergistic effect of biotin and chromium. However, the synergistic effects reported in the McCarty references are in reducing hyperglycemia. Applicants assert that given the teachings of McCarty, the skilled artisan would not expect that the synergistic effects seen in treating hyperglycemia would translate into synergistic effects on raising serum HDL levels. Applicants maintain the position that the two conditions (i.e., hyperglycemia and lowered serum HDL levels) are not necessarily causally related. Nevertheless, even if this were the case, which Applicants maintain it is not, the skilled artisan would not necessarily expect a one-to-one correlation of the effects on different physiological processes and pathways. In other words, given that a composition exhibits a synergistic effect on one physiological process, there is no reasonable expectation that the same composition would have the same synergistic effects on a different physiological process. Accordingly, the skilled artisan would not expect the synergistic elevation in HDL levels, given the teachings of the McCarty references, which deal solely with serum glucose levels.

Applicants' evidence of synergistic results in elevating serum HDL levels is found in Figure 14 of the specification. Figure 14 provides data from experiments in which differing amounts of chromium and biotin were administered to animals. The data illustrates the synergistic effects of chromium and biotin on changes in HDL levels. In particular, Figure 14 illustrates that all combinations of chromium and biotin tested synergistically elevated serum HDL levels (i.e., low chromium/low biotin; low biotin/high chromium; high biotin/low chromium; and high biotin/high chromium), when compared to the same doses of the compounds administered alone. As indicated in paragraph [0110], the nutrients were administered via daily water feeding at a specified dose/kg body weight. Applicants assert that the evidence of synergy is commensurate with the breadth of the claims.

In conclusion, Applicants submit that in view of the totality of the evidence, the Examiner's rejection of the claims as being obvious in view of the cited art is improper. Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 103(a).

## CONCLUSION

In view of the above amendments and remarks, Applicants respectfully maintain that the claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Nov. 28, 2005

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**EXHIBIT A**

# Take Heart

Extensive Cardiovascular Nutrient Support

AllergyResearchGroup®

**Take Heart**
Item no. 73600 (300 grams), Item no. 73830 (900 grams)
**Take Heart II without Hormones**
Item no. 73710 (300 grams), Item no. 73840 (900 grams)

## The Possible Benefits of Take Heart, a Dietary Supplement

- Enhances homocysteine metabolism, helping to control a major cardiovascular risk factor
- Supports regulation of serum viscosity
- Supplies a wide range and quantity of antioxidants, protecting vascular tissue and function
- Nutritionally supports adenosine triphosphate (ATP) production

## Description

Take Heart is a powdered, comprehensive cardiovascular support formula, easy to take, digest and assimilate. (Take Heart II is the same formula but without DHEA and pregnenolone.) Take Heart is synergistically unique, providing nutrients that have been shown to support optimal cardiovascular function. Our food supply nowadays contains significantly reduced nutrient content, even as our requirement for cardiovascular nutritional support has increased, due to greater environmental toxin exposure and the mental and emotional stress inherent in modern life. Take Heart includes all essential nutrients needed for general nutritional support, and a generous supply of specialty agents specifically implicated in cardiovascular wellness. For instance, adenosine triphosphate (ATP), the energy currency of the cells, is crucial to the steady pumping of the heart and the flow of blood, and requires nutrients such as B-vitamins, chromium, magnesium, L-carnitine and CoQ10. Besides supporting general nutrition and ATP production, Take Heart provides nutritional support for other cardiovascular specific functions, such as catabolism and homocysteine metabolism, antioxidant function, maintenance of serum viscosity, and vascular function.

**Vitamins B6, B12,** and **folic acid** work together to help control homocysteine levels. Homocysteine, which is formed during the metabolism of methionine, is a risk factor for the cardiovascular system and the development of atherosclerosis (1-9). Other nutrients supplied by Take Heart that participate in homocysteine regulation are **L-methionine, taurine, phosphatidylcholine,** and **CoQ10.**

**Niacin** has been shown to help optimize LDL and HDL cholesterol levels. Among the successful studies showing niacin's support for circulation is the Coronary Drug Project, which suggested that, for people at risk for heart attacks, niacin may reduce the recurrence rate (10-11).

**Magnesium** provides important support for cardiovascular function, in the areas of control of blood viscosity, relaxation of blood vessel walls, and control of blood pressure. Inadequate magnesium is known to increase the risk of heart disease, and a recent study involving postmenopausal women showed that low magnesium increased the heart rate and the need for oxygen, with decreased cardiovascular function (12-16). It is now known that, compared to healthy people, coronary heart disease sufferers have significantly lower **chromium** levels. Chromium may also help to regulate cholesterol levels (17-18).

**Pregnenolone** is a protective hormone and the precursor to all steroidal hormones, including **DHEA**. Through its anti-inflammatory and especially its antioxidant function, DHEA may help prevent oxidation of lipoproteins, a precursor to atherosclerosis (19-22).

**CoQ10** protects the cellular mitochondria from free radical damage, and helps produce cellular energy. These two functions make CoQ10 especially important for the cardiovascular system. Studies support CoQ10 s ability to help lower high blood pressure, reduce the frequency of angina attacks, strengthen the heart muscle, and increase the quality of life and survivability after congestive heart-failure (23-29). **Lecithin** contains **phosphatidylcholine**, which is known to help regulate the levels and make-up of cholesterol (30).

Besides optimizing LDL and HDL cholesterol levels, **L-carnitine** has been shown to possibly benefit those with angina, irregular heartbeat, cardiomyopathy and peripheral vascular conditions (31-35). **Ornithine** is supplied in higher amounts as a safe way to support L-arginine levels, which as a key component in the nitric oxide pathway, plays a role in vasodilation and cardiovascular function (36-55). **Taurine**, the most plentiful amino acid in the heart, is a key to the contraction and pumping of the heart, and studies have shown it supports both heart function and proper blood pressure (56-58).

**Bromelain, papain** and **pancreatin** can help dissolve fibrin, supporting circulation, serum viscosity, and possibly helping to control angina and inflammation associated with blood clots (59-64).

**Hawthorn Berry Extract** has a long history of use for support of the heart, dating back at least 2000 years. It has been extensively studied and is used in Europe to support

many aspects of circulation, including high or low blood pressure, heartbeat, blood flow, and control of artherosclerotic plaque. It contains bioflavonoids that may dilate blood vessels, possibly helping to control angina attacks. In studies comparing hawthorn extract to either ACE-inhibitors or placebos, it showed better ability to improve heart function (65-70). **Garlic** has been extensively studied for its support of the cardiovascular system. It is known to optimize the make-up and levels of serum cholesterol, to have antioxidant ability, and to protect the cardiovascular system in multiple ways (71-77).

**Flax Seed Oil, EPA,** and **DHA** supply omega-3 fatty acids, which are known to thin the blood, relax and improve elasticity of blood vessels, and help regulate blood pressure and cholesterol and triglyceride levels (78-89).

The heart and blood vessels are especially susceptible to oxidant attack. The **carotene family (alpha, beta,** and **lycopene), CoQ10, Vitamin E** and **tocotrienols, zinc** and **chromium, grape skin extract, glutathione** and **N-acetyl-L-cysteine, lutein** and **lipoic acid** are important antioxidants that help protect the circulatory structures and their functions.

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Serving Size: 20 g (1 Scoop)
Servings Per Container: 15 (300 g) and 45 (900 g)
Amount Per Serving:

| | | | |
|---|---|---|---|
| Calories | 50 | L-Arginine | 480 mg |
| Calories from Fat | 30 | L-Aspartic Acid | 85 mg |
| Total Fat | 3 g | L-Carnitine | 200 mg |
| Saturated Fat | 0.4 g | L-Cystine | 10 mg |
| Cholesterol | 8 mg | L-Glutamine | 600 mg |
| Total Carbohydrates | 4 g | Glycine | 30 mg |
| Dietary Fiber | 1.5 g | L-Histidine | 20 mg |
| Sugars | 1.5 g | L-Isoleucine | 35 mg |
| Protein | 1 g | L-Leucine | 60 mg |
| Vitamin A (as Beta-Carotene) | 2000 IU | L-Lysine | 45 mg |
| Vitamin C (as Ascorbic Acid) | 500 mg | L-Methionine | 160 mg |
| Vitamin D3 | 100 IU | L-Ornithine | 200 mg |
| Vitamin E (as D-alpha-Tocopherol) | 320 IU | L-Phenylalanine | 38 mg |
| Vitamin K | 80 mcg | L-Proline | 38 mg |
| Thiamin (Vitamin B1) | 30 mg | L-Serine | 38 mg |
| Riboflavin (Vitamin B2) | 30 mg | Taurine | 500 mg |
| Niacin | 160 mg | L-Threonine | 28 mg |
| Vitamin B6 | 16 mg | L-Tyrosine | 220 mg |
| Folic Acid | 320 mcg | L-Valine | 40 mg |
| Vitamin B12 | 80 mcg | N-Acetyl-L-Cysteine | 200 mg |
| Biotin | 240 mcg | Glutathione (reduced) | 100 mg |
| Pantothenic Acid | 100 mg | Pancreatin | 10 mg |
| Calcium (as Calcium Citrate/Gluconate) | 280 mg | Papain | 16mg |
| Magnesium (as Magnesium Glycinate/Gluconate) | 160 mg | Bromelain | 20 mg |
| Zinc (as Zinc Arginate) | 12 mg | Grape Skin Extract | 200 mg |
| Selenium (as Sodium Selenite/Selenomethione) | 80 mcg | Hawthorne Berry Extract | 200 mg |
| Copper (as Copper Glycinate) | 0.8 mg | Garlic (1% Allicin) | 100 mg |
| Manganese (as Manganese Glycinate) | 1 mg | Lutein | 2 mg |
| Chromium (as Chromium Picolinate) | 160 mcg | Lycopene | 0.4 mg |
| Molybdenum (as Sodium Molybdate) | 40 mcg | Tocotrienols | 20 mg |
| Potassium (as Potassium Gluconate) | 40 mg | Trimethylglycine | 200 mg |
| Boron (as Boron Citrate) | 0.4 mg | Methylsulfonylmethane | 80 mg |
| Vanadium (as Vanadium Pentoxide) | 80 mcg | Eicosapentaenoic Acid (EPA) | 145 mg |
| Alpha-Carotene | 8 mg | Docosahexaenoic Acid (DHA) | 95 mg |
| Pregnenolone* | 6 mg | Gamma-Linolenic Acid | 90 mg |
| Dehydroepiandrosterone (DHEA)* | 6 mg | Linolenic Acid | 220 mg |
| Coenzyme Q10 | 60 mg | Oleic Acid | 85 mg |
| Inositol | 50 mg | Palmitic Acid | 65 mg |
| Choline | 50 mg | Stearic Acid | 25 mg |
| Para-Aminobenzoic Acid (PABA) | 20 mg | Flax Seed Oil | 400 mg |
| Lecithin (26% Phosphatidylcholine) | 500 mg | Lipoic Acid | 30 mg |
| L-Alanine | 30 mg | | |

*Not included in Take Heart II without Hormones
Other ingredients: Oat bran, honey powder, rice bran, psyllium bran, apple fiber, lemon flavor, stevia.

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**Suggested use:** As a dietary supplement, 1 to 2 level scoops daily, or as directed by a healthcare practitioner. Take Heart is best mixed in a jar or shaker with 2 to 3 ounces of juice or other beverage such as soy milk. Drink immediately and follow with several ounces more of liquid.

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**References available upon request.**

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March - 2003

**EXHIBIT B**

# Vitamin C (Ascorbic Acid)



## Overview

Vitamin C is a water-soluble vitamin needed for the growth and repair of tissues in all parts of the body. It is necessary to form collagen, an important protein used to make skin, scar tissue, tendons, ligaments, and blood vessels. Vitamin C is essential for the healing of wounds, and for the repair and maintenance of cartilage, bones, and teeth.

Vitamin C is one of many antioxidants. Vitamin E and beta-carotene are two other well known antioxidants. Antioxidants are nutrients that block some of the damage caused by free radicals, which are by-products that result when our bodies transform food into energy. The build up of these by-products over time is largely responsible for the aging process and can contribute to the development of various health conditions such as cancer, heart disease, and a host of inflammatory conditions like arthritis. Antioxidants also help reduce the damage to the body caused by toxic chemicals and pollutants such as cigarette smoke.

Vitamin C deficiency can lead to dry and splitting hair; gingivitis (inflammation of the gums) and bleeding gums; rough, dry, scaly skin; decreased wound-healing rate, easy bruising; nosebleeds; weakened enamel of the teeth; swollen and painful joints; anemia; decreased ability to ward off infection; and, possibly, weight gain because of slowed metabolic rate and energy expenditure. A severe form of vitamin C deficiency is known as scurvy, which mainly affects older, malnourished adults.

The body does not manufacture vitamin C on its own, nor does it store it. It is therefore important to include plenty of vitamin C-containing foods in one's daily diet. Large amounts of vitamin C are used by the body during any kind of healing process, whether it's from an infection, disease, injury, or surgery. In these cases extra vitamin C may be needed.

## Uses

Low levels of vitamin C have been associated with a variety of conditions including hypertension, gallbladder disease, stroke, some cancers, and atherosclerosis (the build up of plaque in blood vessels that can lead to heart attack and stroke; conditions that are caused by atherosclerotic build up are often collectively referred to as cardiovascular diseases). Eating adequate amounts of vitamin C in the diet (primarily through lots of fresh fruits and vegetables) may help reduce the risk of developing some of these conditions. There is little evidence, however, that vitamin C supplements can cure any of these diseases.

As an anti-oxidant, vitamin C plays an important role in protecting against the following:

**Heart Disease**

Results of scientific studies regarding the benefit of vitamin C for heart disease or stroke is somewhat confusing. While not all of the studies agree, some information suggests that vitamin C may help protect blood vessels from the damaging effects that lead to or result from the presence of atherosclerosis.

For example, those with low levels of vitamin C may be more likely to have a heart attack, stroke, or peripheral artery disease, all potential outcomes of atherosclerosis. Peripheral artery disease is the term used to describe atherosclerosis of the blood vessels to the legs. This can lead to pain with walking, known as intermittent claudication.

In terms of damage that can cause atherosclerosis, some studies have shown that vitamin C helps prevent oxidation of LDL (bad) cholesterol – a process that contributes to plaque buildup in the arteries.

Under most circumstances, dietary vitamin C is adequate for protecting against the development of or consequences from cardiovascular disease. If you have low levels of this nutrient, however, and find it difficult to obtain through dietary sources, a knowledgeable healthcare provider may recommend vitamin C supplements.

**High Cholesterol**

Information from several studies, involving only small numbers of people, suggest that vitamin C (3 glasses of orange juice per day or up to 2000 mg per day as a supplement) may help decrease total and LDL cholesterol and triglycerides, as well as increase HDL levels (the good kind of cholesterol). Studies evaluating larger groups of people would be helpful in determining how accurate these preliminary research results are and to whom this potential benefit applies.

**High Blood Pressure**

Free radicals, the damaging by-products of metabolism mentioned earlier, are associated with higher blood pressure in studies of animals and people. Population based studies (which involve observing large groups of people over time) suggest that people who eat foods rich in antioxidants, including vitamin C, are less prone to high blood pressure than people without these nutritious foods in their diet. For this reason, many clinicians recommend foods rich in vitamin C, particularly if you are at risk for high blood pressure. In fact, the diet most frequently recommended for treatment and prevention of hypertension, known as the DASH (Dietary Approaches to Stop Hypertension) diet advocates lots of fruits and vegetables, which are loaded with antioxidants.

**Common Cold**

Despite the popular belief that vitamin C can cure the common cold, the scientific evidence supporting this conviction is limited. There have been a few studies suggesting that taking large doses of vitamin C supplements at the onset of cold or flu symptoms, or just after exposure to one of these viruses, can shorten the duration of the cold or ward it off altogether. However, the majority of studies, when looked at collectively, lead researchers to conclude that vitamin C does not prevent or treat the common cold. Some experts suggest that vitamin C may only be useful in case of a cold if you have low levels of this nutrient to begin with. Another possibility is that the likelihood of success may be very individual – some improve, while others do not. If you are amongst the 67% of people who believe that vitamin C is helpful for your colds, there may be power in your conviction. In other words, your experience is probably more important than what the research is stating. Talk to your doctor about any pros and cons with regards to using vitamin C during cold and flu season.

**Cancer**

While the precise role of vitamin C in preventing cancer remains controversial, results of many population based studies (evaluating groups of people over time) imply that foods rich in vitamin C may be associated with lower rates of cancer, including skin cancer, cervical dysplasia (changes to the cervix which may be cancerous or precancerous, picked up by pap smear), and, possibly, breast cancer. At best, however, particularly for breast cancer, the specific connection of vitamin C and cancer prevention is weak. This is mainly because protection comes from eating foods, such as fruits and vegetables, which contain many beneficial nutrients and antioxidants, not only vitamin C.

Also, there is no evidence that taking large doses of vitamin C once diagnosed with cancer will help your treatment. In fact, there is concern that large doses of antioxidants from supplements could interfere with chemotherapy medications. Much more research in the area of antioxidants and cancer treatment is needed.

**Osteoarthritis**

Vitamin C is essential for normal cartilage. Plus, free radicals can be produced in the joints and have been implicated in many degenerative changes in the aging body, including destruction of cartilage and connective tissue that lead to arthritis. Antioxidants appear to offset the damage caused by free radicals. Although further evidence is needed to substantiate these claims, studies of groups of people observed over time suggest that vitamin C, as well as vitamin E, may help to reduce the symptoms of OA.

**Obesity and Weight Loss**

Studies suggest that obese individuals may have lower vitamin C levels than nonobese individuals. Researchers speculate that insufficient amounts of vitamin C may contribute to weight gain by decreasing metabolic rates and energy expenditures. Many sensible weight loss programs will be sure to include foods rich in vitamin C, such as plenty of fruits and vegetables.

**Cataracts**

Studies have shown that vitamin C may slow or even stop the progression of cataracts in the elderly. A recent study, for example, of women from the Nurses' Health Study (a very large, important study that has followed women over many years) showed that women under 60 years of age who had high dietary intake of vitamin C or who had used vitamin C supplements for 10 years or more had significantly reduced chances of developing cataracts.

**Age-related Macular Degeneration**

Vitamin C works together with other antioxidants like selenium, beta-carotene, and vitamin E to protect the eyes against developing macular degeneration. This is a painless, degenerative eye disease that affects more than 10 million Americans. It is the leading cause of legal blindness in persons over the age of 55 in the United States. While complete blindness does not occur in most people with the disorder, macular degeneration often interferes with reading, driving, or performing other daily activities.

While not all research agrees, antioxidants, including vitamin C, primarily from dietary sources may help prevent macular degeneration. Many qualified clinicians will recommend a combination of these nutrients for treating or preventing this serious and frustrating eye disorder.

**Diabetes**

Vitamin C may be helpful for people with diabetes in a number of ways. First, some studies suggest that people with diabetes have high levels of free radicals (the damaging metabolic by-products, mentioned earlier, associated with many chronic illnesses) and low levels of antioxidants, including vitamin C. This imbalance may

contribute to the fact that those with diabetes are at greater risk for developing conditions such as high cholesterol and atherosclerosis.

Secondly, insulin (which is low in type 1 diabetics and does not function properly in type 2 diabetics) helps cells in the body take up the vitamin C that they need to function properly. At the same time, lots of circulating blood sugar (glucose), as is often the present in diabetics, prevents the cells from getting the vitamin C that they need, even if eating lots of fruits of vegetables. For this reason, taking extra vitamin C in the form of supplements may be helpful in those with diabetes.

**Alzheimer's Disease and other types of Dementia**
While the evidence is somewhat stronger for another important antioxidant, namely vitamin E, vitamin C may help prevent the development of Alzheimer's disease. It may also improve cognitive function in dementia from causes other than Alzheimer's (such as multiple strokes). The use of these antioxidants for improving cognitive ability in those who already have dementia of the Alzheimer's type has not been well tested to date.

**Other**
Although the information is somewhat limited, studies suggest that vitamin C may also be helpful for:

- Boosting immune system function
- Maintaining healthy gums
- Relieving eye pressure in those with glaucoma
- Improving visual clarity for those with uveitis (an inflammation of the middle part of the eye)
- Slowing progression of Parkinson's disease
- Treating allergy-related conditions, such as asthma, eczema, and hay fever (called allergic rhinitis)
- Relieving pain from pancreatitis; vitamin C levels are often low with this condition
- Reducing effects of sun exposure, such as sunburn or redness (called erythema) and even, possibly, skin cancer
- Alleviating dry mouth, particularly from antidepresant medications (a common side effect from these drugs)
- Healing burns and wounds

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## Dietary Sources

Since vitamin C is not produced by the body, it must be obtained from fruits and vegetables. Some excellent sources of vitamin C are oranges, green peppers, watermelon, papaya, grapefruit, cantaloupe, strawberries, kiwi, mango, broccoli, tomatoes, brussels sprouts, cauliflower, cabbage, and citrus juices or juices fortified with Vitamin C. Raw and cooked leafy greens (turnip greens, spinach), red and green peppers, canned and fresh tomatoes, potatoes, winter squash, raspberries, blueberries, cranberries and pineapple are also rich sources of Vitamin C. Vitamin C is sensitive to light, air, and heat, so it is best to eat fruits and vegetables raw, or minimally cooked in order to retain their full vitamin C content.

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## Available Forms

You can purchase either natural or synthetic vitamin C, also called ascorbic acid, in a wide variety of forms. Tablets, capsules, and chewables are probably the most popular, but vitamin C also comes in powdered

crystalline, effervescent, and liquid forms. Vitamin C can be purchased in dosages ranging from 25 mg to 1,000 mg.

"Buffered" vitamin C is also available if you find that regular ascorbic acid upsets your stomach. An esterified form of vitamin C is also available, which tends to be better tolerated by people who are prone to heartburn or have a sensitive stomach.

Some vitamin C supplements contain bioflavonoids, which appear to enhance absorption and utilization of ascorbic acid.

There is concern about tooth enamel erosion occurring from the acid content of chewable vitamin C.

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## How to Take It

Vitamin C is not stored in the body, so it must be replaced as it gets used. The best way to take supplements is with meals two or three times per day, depending on the dosage. Some studies suggest that adults should take between 250 mg and 500 mg twice a day for maximum benefit. A knowledgeable healthcare provider should be consulted before taking more than 1,000 mg of vitamin C on a daily basis and before giving vitamin C to a child.

Daily intake of dietary vitamin C (according to the U.S. RDA), are listed below.

**Pediatric**

- Neonates 1 to 6 months: 30 mg
- Infants 6 to 12 months: 35 mg
- Children 1 to 3 years: 40 mg
- Children 4 to 6 years: 45 mg
- Children 7 to 10 years: 45 mg
- Children 11 to 14 years: 50 mg
- Adolescent girls 15 to18 years: 65 mg
- Adolescent boys 15 to18 years: 75 mg

**Adult**

- Men over 18 years: 90 mg
- Women over 18 years: 75 mg
- Breastfeeding women: first 6 months: 95 mg
- Breastfeeding women: second 6 months: 90 mg

Because smoking depletes vitamin C, people who smoke generally need an additional 35 mg/day.

The doses recommended to prevent or to treat many of the conditions mentioned in the Uses section is often between 500 and 1,000 mg per day.

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## Precautions

Because of the potential for side effects and interactions with medications, dietary supplements should be taken only under the supervision of a knowledgeable healthcare provider.

It is important to drink plenty of fluids when taking supplemental vitamin C because it has a diuretic effect.

Most commercially available vitamin C is derived from corn. People sensitive to corn should look for alternative sources, such as sago palm.

Vitamin C increases the amount of iron absorbed from foods. This may be helpful for people who have low blood iron levels. However, people with hemochromatosis should not take vitamin C supplements because of enhanced accumulation of non-heme iron in the presence of this vitamin.

During periods of stress (either emotional or physical), urinary excretion of vitamin C is increased. Extra vitamin C through vitamin C rich foods as well as supplements is often recommended to keep the immune system working properly during these times.

While vitamin C is generally non-toxic, in high doses (more than 2,000 mg daily) it can cause diarrhea, gas, or stomach upset. Those who have kidney problems should check with a healthcare provider before taking vitamin C supplements. Infants born to mothers taking 6,000 mg or more of vitamin C may develop rebound scurvy due to a sudden drop in daily intake. As described earlier, scurvy is a condition caused by extreme vitamin C deficiency. See earlier explanation for the possible symptoms of vitamin C deficiency.

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## Possible Interactions

If you are currently being treated with any of the following medications, you should not use vitamin C supplements without first talking to your healthcare provider.

**Aspirin and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)**
Very limited research suggests that vitamin C may protect the stomach and intestines against injury from NSAIDs such as ibuoprofen. On the other hand, high doses of vitamin C (equal to or greater than 500 mg per day) may raise the blood levels of aspirin and other acidic medications.

**Acetominophen**
Vitamin C may decrease excretion of acetaminophen (a medication sold over the counter for pain and headache) in the urine, which may increase blood levels of this medication.

**Diuretics, Loop**
Animal studies suggest that vitamin C may amplify the effects of furosemide, which belongs to a class of medications known as loop diuretics.

**Beta-blockers for high blood pressure**
Vitamin C may decrease the absorption of propranolol, a medication that belongs to a class known as beta-blockers used for high blood pressure and other heart-related conditions. If taking vitamin C and a beta-blocker, therefore, it is best to take them at different times of the day.

**Cyclosporine**
Cyclosporine, a medication used for the treatment of cancer, may reduce blood levels of vitamin C.

**Nitrate Medications for heart disease**

The combination of vitamin C with nitroglycerin, isosorbide dinitrate, or isosorbide mononitrate reduces the occurrence of nitrate tolerance. Nitrate tolerance is when the body builds up a tolerance to the medicine so that it no longer has its desired effect. People taking nitrate-containing medications generally follow a 12 hours on, 12 hours off schedule to avoid this tolerance. Studies suggest that taking vitamin C along with nitrate medications may reduce the development of this tolerance.

**Tetracycline**

There is some evidence that taking vitamin C with the antibiotic tetracycline may increase the levels of this medication.

**Warfarin**

There have been rare case reports of vitamin C interfering with the effectiveness of this blood thinning medication. In recent follow up studies, no such association has been found with doses of vitamin C up to 1,000 mg per day. Because of these much earlier reports, however, some conservative clinicians suggest not exceeding RDA values of vitamin C (see earlier section entitled How To Take It). Whether taking recommended dietary amounts or larger quantities of vitamin C, anyone on warfarin must have their bleeding time measured regularly and followed closely using a value called an INR, measured at your doctor's office. If you take this blood thinner, any time you make a change to your diet, medications, or supplements, you must notify your physician.

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Regulation of hyperglycemia and **dyslipidemia** by exogenous **L-arginine** in diabetic rats.

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The effect of **L-arginine** on the pattern of lipids and lipoproteins in normal and diabetic rats was studied. Three groups of 48 rats were studied during 12 days and compared with a control group (Group I, **n** = 5). Group I consisted of normal rats not treated with **L-arginine**. Group II. Normal rats treated with **10** mM **L-arginine** (i.p.). Group III. Diabetic rats (alloxan 120 mg/kg, i.p.) not treated (diabetic control). Group IV. Diabetic rats treated with **10** mM **L-arginine** (i.p.). The rats of each group were divided in subgroups of four each. Rats were anesthetized and blood was taken from aorta to determine glucose, triglycerides, cholesterol, total lipids, and low (LDL) and high density lipoproteins (HDL) and their corresponding apoproteins (Apo A-I and Apo B-100). We observed that the alloxan concentration used in this study reproduces the clinical manifestations of disease including hyperglycemia (from 132.5 +/- 7.6 to 544.3 +/- 16.9 mg/**dL**) in 96 h. As a consequence the levels of triglycerides, cholesterol, total lipids, and LDL and its apoprotein Apo B-100 were increased, whereas HDL and its apoprotein Apo A-I were diminished. The **L-arginine** injection tends to normalize the glycemia from 24 h; similarly, hyperlipidemia (triglycerides from 924.7 +/- 220.1 to 68.5 +/- 8.4 mg/**dL**, cholesterol from 107.7 +/- 0.6 to 64.5 +/- 4.2 mg/**dL**, LDL from 24.2 +/- 2.5 to 8.0 +/- 2.9 mg/**dL**) was also diminished. These results suggest that the beneficial effect of **L-arginine** administration on serum glucose values and lipid levels in diabetic rats can be mediated by polyamine formation, although the effect of **L-arginine** on insulin release as observed by other authors is not discarded.

| Major Subject Heading(s) | Minor Subject Heading(s) | CAS Registry / EC Numbers |
|---|---|---|
| • **Arginine** [metabolism]<br>• Hyperglycemia<br>• Hyperlipidemia | • Animals<br>• Aorta [metabolism]<br>• Apolipoprotein A-I [metabolism]<br>• Apolipoproteins B [metabolism]<br>• Blood Glucose [metabolism]<br>• Body Weight<br>• Cholesterol [metabolism]<br>• Diabetes Mellitus, Experimental<br>• Glucose [metabolism]<br>• Lipoproteins, HDL [metabolism]<br>• Lipoproteins, LDL [metabolism]<br>• Rats, Sprague-Dawley<br>• Rats<br>• Time Factors<br>• Triglycerides [metabolism] | • 0 (Apolipoprotein A-I)<br>• 0 (Apolipoproteins B)<br>• 0 (Blood Glucose)<br>• 0 (Lipoproteins, HDL)<br>• 0 (Lipoproteins, LDL)<br>• 0 (Triglycerides)<br>• 0 (apolipoprotein B-100)<br>• 50-99-7 (Glucose)<br>• 57-88-5 (Cholesterol)<br>• 74-79-3 (**Arginine**) |

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# *COCHRAN FOUNDATION OF MEDICAL RESEARCH Specializing in Cardiovascular and Autoimmune Diseases · Clinical Test Trials Operating in United States and India · Eight Medical Doctors and Biochemist on Staff with over 200 years of medical training and experience*

- **L-CARNITINE** Report up-date on June 8, 1997

L-Carnitine supports fat metabolism for cardiovascular health. L-Carnitine transports long-chain fatty acids into the mitochondria of each cell. The fats are then broken down into energy, which is used to fuel the cardiovascular and Muscular Systems of the body.

When insufficient levels of L-Carnitine are present, then too few fatty acids are transported into the cell. Instead of being effectively utilized as fuel, the fat builds up in the bloodstream and as a result, lethargy, muscle weakness, and fatigue will happen. Long term cardiovascular health and maintenance is sacrificed in the process.

L-Carnitine is found naturally in red meat (So is Coenzyme Q-10) so the supplementation for vegetarians is of the uppermost importance. Especially since as we age the level of this amino acid drops throughout the aging process. In addition, as the body ages, it becomes less efficient at metabolizing nutrients, so that the elderly will benefit greatly from L-Carnitine supplements.

By enabling the body to operate more efficiently by utilizing fat for energy, L-Carnitine enhances Energy, Stamina and metabolic rate levels. Clinical studies have shown an increase in exercise and cardiovascular tolerance and a decrease in fatigue in cardiovascular disease patients who took supplemental L-Carnitine. By ensuring adequate utilization of fatty acids to fuel the heart and other essential organs, L-Carnitine is able to help combat fatigue, enhance the body's energy levels and help build lean muscle mass and increase stamina and the metabolic rate of the body.

Carnitine is important in the regulatory effect upon fat metabolism in the heart and skeletal muscles. Under our medical trials it has been shown to stimulate fat metabolism and it assists in the clearance of triglycerides and fatty acids from the blood stream. In human metabolism, the amino acid transport system is utilized in the transfer of fatty acids across the cell membrane and on to the mitochondria. It is here at the mitochondria level; once the fatty acids are finally delivered that they can be used as an efficient source of fuel for generating energy on a cellular level for the body. Carnitine is not a vitamin but is an amino acid, and is found only in animal muscle tissue and organs. Carnitine can't be source from vegetable or fruit diets. The highest concentration level of Carnitine found in the human body is in one's internal organs and skeletal muscle groups.

We have found that there is a great different between men and woman for the internal need for this amino acid. Men have and need to have a much a higher blood level of Carnitine then women. This is because the highest level of Carnitine is found in the epididymis of the testes in males. Carnitine is necessary for energy metabolism with in the sperm for proper mobility, to strengthen and enhance the sperm metabolic rate for fertilization. In humans or mammals that have low to very low Carnitine blood level, sperm just doesn't have the proper strength levels to supply them with the required energy to complete their course to the female egg in waiting.

Our Medical trials are currently underway and we have experienced some very striking results with regards to both cardiovascular diseases and Fertility. Additional information will be added when our trial data is reviewed and complied.

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These Links are from our old site please visit our new Web Site WWW.Cochranfoundation.org

# Does Oral Folic Acid Lower Total Homocysteine Levels and Improve Endothelial Function in Children With Chronic Renal Failure?

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***Background***—Accelerated vascular disease is common in chronic renal failure (CRF) and accounts for significant mortality and morbidity. Elevated homocysteine levels may contribute by an effect on endothelial function.

***Methods and Results***—We performed a double-blind placebo-controlled randomized crossover trial of folic acid at 5 mg/m$^2$ in 25 normotensive children 12±3 (7 to 17) years of age with CRF (glomerular filtration rate 26.8±13.2 mL/min per 1.73 m$^2$) of noninflammatory etiology. Each subject underwent two 8-week periods of folic acid and placebo separated by an 8-week washout period. The effect of folic acid on homocysteine levels, LDL oxidation, and both endothelial-dependent and -independent vascular function were measured. After oral folic acid, serum folate levels rose from 11.7±4.25 to 635±519 μg/L ($P$=0.001), red cell folate levels rose from 364±195 to 2891±2623 μg/L ($P$<0.001), and total homocysteine levels fell from 10.28±4.16 to 8.62±2.32 μmol/L ($P$=0.03). In addition, there was a significant improvement in flow-mediated dilatation (FMD) (endothelial-dependent dilatation) from 7.21±2.8% to 8.47±3.01% ($P$=0.036) with no change in response to glyceryl trinitrate (endothelial-independent dilatation). There was no significant change in FMD or glyceryl trinitrate during the placebo phase. There was, however, no significant difference in final FMD after placebo or folic acid. Lag times for LDL oxidation were prolonged during the treatment phase (58.4±18.7 to 68.1±25.9 minutes, $P$=0.01).

***Conclusion***—Folic acid supplementation in children with CRF may improve endothelial function with an increased resistance of LDL to oxidation. (***Circulation.*** **2002;105:1810-1815.**)

**Key Words:** homocysteine ■ folic acid ■ renal failure ■ endothelium

Premature atherosclerosis is a major cause of morbidity and mortality in adults with chronic renal failure (CRF). This may be due not only to the increased incidence of classic risk factors such as glucose intolerance, hypertension, and dyslipidemia but also to a direct adverse effect of CRF.

We have demonstrated endothelial dysfunction, a key early event in atherogenesis, in children with CRF without additional classic risk factors or clinical vascular disease.[1,2] One possible mechanism for endothelial damage in CRF is the presence of high circulating levels of homocysteine. Homocysteine is a sulfur-containing amino acid formed as an intermediate during the metabolism of methionine, which has been shown in population studies to be an independent risk factor for both vascular disease[3,4] and myocardial infarction.[5,6] In CRF, homocysteine is also an independent risk factor,[7] and in dialysis patients, hyperhomocystinemia is more prevalent than traditional cardiovascular risk factors.[8] Homocysteine may, therefore, contribute to aggressive "accelerated atherosclerosis" in CRF. In vitro and in vivo studies suggest that homocysteine causes endothelial dysfunction either directly or via intermediate reactions by increasing oxidized LDL levels.[9] Even modestly elevated homocysteine levels may be particularly damaging in the presence of the atherogenic risk profile of CRF.[10]

Folic acid has been shown to lower homocysteine levels in several populations and can improve brachial artery endothelial function.[11–13] In CRF, there appears to be relative resistance to folic acid, but supplementation in adults with doses of 5 to 15 mg/day can decrease homocysteine levels by as much as 40% to 50%.[14] The impact on endothelial function has, however, been disappointing.[15–17]

We report the use of high-resolution ultrasound to study the effect of folic acid supplementation on homocysteine and vascular function in children with moderate to severe CRF. Children were selected specifically both to reduce the influence of confounding factors and, thus, provide a clinical

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model of uremic influences on the arterial wall and to determine whether early intervention might have greater vascular benefits than those seen in adults.

## Methods

### Subjects

Twenty-five children (11 girls and 14 boys, mean age 12±3 years [range 7 to 17 years]) with CRF (glomerular filtration rate <50 mL/min per 1.73 $m^2$) were recruited from the outpatient department at Great Ormond Street Hospital for Children. Twenty-four children had congenital structural causes of CRF, and 1 child had an acquired (cortical necrosis) cause of CRF. Sample size was based on an estimated benefit of 2% in flow-mediated dilatation (FMD) with 80% power and a 5% significance level. We excluded children who were smokers, hypertensive, diabetic or nephrotic, or on vasoactive medication or dialysis. No child received folic acid supplementation or vitamins (apart from activated vitamin D) before the study. The local research ethics committee approved the study, and informed consent was obtained from the parents or guardian or from the patient in those >16 years of age.

### Study Design

We performed a randomized, placebo-controlled, double-blinded, crossover trial with two 8-week treatment periods separated by an 8-week washout period. Folic acid was given at a dose of 5 mg/$m^2$ surface area (Special Products Ltd, Addlestone, Surrey, UK, who also prepared the placebo).

Children were evaluated at the start and the end of each treatment period. At each visit, supine blood pressure was recorded, blood was taken (after a 6-hour fast), and vascular function was assessed.

### Assessment of Vascular Function

Endothelial function was determined by recording the dilator response of the brachial artery to increased blood flow generated during reactive hyperemia (FMD). Subjects lay supine in a temperature-controlled laboratory (22°C to 25°C). The brachial artery was scanned in longitudinal section with a 7-MHz linear array transducer and an XP 128/10 (Acuson), magnified using a resolution box function and gated with the R wave of the ECG. End-diastolic images of the vessel were acquired every 3 seconds using data acquisition software (Information Integrity) throughout the whole study and were stored off-line for later analysis. Arterial diameter over a 1- to 2-cm segment was determined for each image using automatic edge detection software (Information Integrity). Analysis was performed by an experienced vascular technician blinded to the phase of the study. With pulse-wave Doppler, blood flow was recorded continuously throughout the study and was expressed as the velocity time integral (area under the blood velocity/time curve for a complete cardiac cycle). Baseline recordings of arterial diameter were made for 1 minute before inflation of a blood pressure cuff placed distal to the site of arterial imaging. Recording continued for 5 minutes during cuff inflation to 300 mm Hg and for 4 minutes after deflation. The time point of maximum change in diameter was also recorded. Endothelium-independent dilatation of the brachial artery was assessed by measuring the dilator response to a 25-μg dose of the nitric oxide (NO) donor, glyceryl trinitrate (GTN) given sublingually. This elicited vascular dilatation of the same magnitude as that of the endothelium-dependent flow stimulus. Results are expressed as both percentage and absolute maximum change in vessel diameter.

### Laboratory Assays

Full blood count, urea, creatinine, bicarbonate, and electrolytes were measured (Vitros 750, Ortho-Clinical Diagnostics). Fasting lipid analyses were performed for total cholesterol, HDL, and triglycerides with colorimetric assays (Vitros 750, Ortho-Clinical). LDL values were calculated, and LDL subfractions were measured with high-resolution polyacrylamide gel electrophoresis (Quantimetrix), reported as the ratio of less dense to more dense (LDL1+2:LDL3+4+5). LDL lag times were measured by isolating LDL with density-gradient ultracentrifugation and were desalted by gel filtration. Oxidation was promoted with copper, conjugated diene production was monitored, and lag times were generated.[18] Total serum antioxidant activity was measured with a chemiluminescent assay. This is based on a catalyzed oxidation of luminol (chemiluminescent substrate) by hydrogen peroxide, which generates free radicals. The duration of suppression of this reaction by the subject's serum is a measure of its total antioxidant capacity. This is compared against a standard curve created by a calibrant and provides a rapid, reproducible measure of antioxidant defense in biological fluids.[19] Serum and red cell folate levels were determined with a radioimmunoassay (Abott IMx) with a normal range for serum folate of 2 to 20 μg/L and for red cell folate of 150 to 650 μg/L. Plasma total (free and bound) homocysteine was measured with a competitive fluorescence polarization immunoassay (normal range 4.4 to 13.7 μmol/L for adults, Abbot IMx).

**TABLE 1. Physical and Biochemical Characteristics at Entry of the 23 Children Who Completed the Study**

| | Mean±SD |
|---|---|
| Age, years | 11.5±3 |
| Sex, male:female | 13:10 |
| Height, cm | 144.3±17.9 |
| Weight, kg | 40.9±14.7 |
| Systolic/diastolic blood pressure, mm Hg | 110±10/67±9 |
| Glomerular filtration rate (NR: 80–120 mL/min per 1.73 $m^2$) | 28.3±12.7 |
| Serum creatinine (NR: 40–102 μmol/L) | 229±193 |
| Total homocysteine (NR: 4.4–13.7 μmol/L) | 9.85±3.57 |
| Serum total cholesterol (NR: 3.1–5.4 mmol/L) | 4.74±1.05 |
| Serum triglycerides (NR: 0.4–1.4 mmol/L) | 1.66±0.65 |
| Hemoglobin (NR: 13–16 g/dL) | 12.8±1.49 |

Normal range (NR) is given where appropriate.

### Analysis

Each subject served as their own control. The data were tested for normality with the Shapiro-Wilks and the modified Kolmogorov-Smirnov tests. The data were analyzed in 2 ways. First, change in FMD (post-treatment value minus pretreatment value) on folic acid or placebo was compared with a paired $t$ test. Second, final FMD after folic acid and after placebo were compared with ANCOVA.[20] All descriptive data are expressed as group mean±SD, and significance is interpreted as $P<0.05$.

## Results

The clinical and biochemical characteristics of the study group are shown in Table 1. Twenty-three children completed the study. One child was transferred to peritoneal dialysis, and 1 child received a renal transplant.

### Effect of Folic Acid

There was no effect of folic acid on hemoglobin or renal function (Table 2). At entry to the study, serum folate (13.7±3.58 μg/L) and red cell folate levels (334±202 μg/L) were normal. Folic acid produced a significant increase in both serum folate (11.7±4.25 to 635±519 μg/L, $P=0.001$) and red cell folate (364±195 to 2891±2623 μg/L, $P<0.001$) levels during the treatment period.

During placebo, there was no change in serum or red cell folate levels when the placebo phase preceded the folic acid

**TABLE 2. Biochemical Responses to Folic Acid and Placebo**

| | Baseline | After Placebo | | Baseline | After Folic Acid | |
|---|---|---|---|---|---|---|
| Serum folate (NR: 3–20 μmol/L) | 17.0±8.9 | 12.4±6.0 | NS | 13.1±8.8 | 635±519 | 0.001 |
| Red cell folate (NR: 150–650 μmol/L) | 596±468 | 405±168 | 0.02 | 364±195 | 2891±2623 | 0.0004 |
| Total homocysteine (NR: 4.4–13.7 μmol/L) | 9.02±2.19 | 9.84±2.74 | NS | 10.28±4.16 | 8.62±2.32 | 0.03 |
| Total antioxidant activity (normal: 440 μtrolox Eq) | 188±66 | 216±74 | NS | 203±80 | 207±74 | NS |
| LDL lag times (normal: 60 min) | 62.8±17 | 63.2±13 | NS | 58.4±18 | 68.4±25 | 0.001 |

Results are given as mean±SD. Normal ranges (NR) in brackets. NS indicates not significant.

phase (13.6±4.6 to 10.68±5.76 μg/L, and 348±244 to 351±127 μg/L, $P$=ns). However, in the children who received placebo after folic acid, the serum folate changed from 20±9.9 to 14.01±6.08 μg/L ($P$=ns) and the red cell folate changed from 820±517 to 470±185 μg/L ($P$=0.02) during the placebo phase. These postplacebo levels were higher at the end of the study than at entry, which suggested a carry over effect for red cell folate.

### Homocysteine Levels

Homocysteine levels at entry to the study were greater (9.85±3.57 μmol/L) than published data on normal children (Table 2). There was a significant fall in total homocysteine levels after folic acid (10.28±4.16 mol/L to 8.62±2.32 mol/L, $P$=0.03) but not in the placebo phase (9.02±2.19 to 9.84±2.7 μmol/L, $P$=0.3).

### Lipid Analysis

Baseline total cholesterol levels were within the normal range (4.74±1.05 mmol/L), and there was no significant change with treatment or placebo. Triglycerides were elevated above the normal range (1.66±0.65 mmol/L) and were unchanged after folic acid or placebo (Table 2). HDL and LDL cholesterol were within the normal range at baseline (1.36±0.36 mmol/L [normal range 0.93 to 1.94] and 2.7±0.8 mmol/L [normal range 1.63 to 3.63], respectively) and did not change significantly with either treatment or placebo.

### Oxidant Stress

Baseline values for LDL lag times were within the normal range (Table 2). There was a significant increase in LDL lag times after folic acid (58.4±18.7 to 68.1±25.9 minutes, $P$=0.01) compared with placebo (62.8±17.4 to 63.2±13.3 minutes $P$=0.92), which suggests that folic acid supplementation reduced susceptibility of LDL to oxidation. Ratios of LDL to HDL (25±37 to 24±34, $P$=ns) remained unchanged during treatment and placebo phases (22±32 to 30±36, $P$=ns) as did total serum antioxidant activity (204±80 to 208±74 on treatment vs 188±65 to 216±74 μtrolox Eq on placebo, $P$=ns).

### Effect of Folic Acid on Vasomotor Function

There was no significant change in baseline arterial diameter, baseline arterial flow, or peak reactive hyperemia after folic acid or placebo (Table 3).

### Endothelial-Dependent Dilatation: FMD

A significant improvement in FMD, expressed as percentage and absolute change in vessel diameter (7.21±2.81% to 8.47±3.01%, $P$=0.036, and 0.217±0.106 cm to 0.252±0.081 cm, $P$=0.47), was seen after folic acid, which was not seen after placebo (8.20±3.41% to 8.80±4.01%, $P$=0.44, and 0.244±0.102 cm to 0.276±0.104 cm, $P$=0.14). There was, however, no statistically significant difference in post-treatment FMD after placebo or folic acid ($P$=ns). Mean time of maximum dilatation after cuff release was not significantly different before or after treatment phases (pre-placebo 54±16 seconds, pre-folic acid 59±13 seconds, postplacebo 65±19 seconds, and post–folic acid 66±17 seconds). No carry over or period effect on FMD was detected ($P$=0.2 and $P$=0.17, respectively).

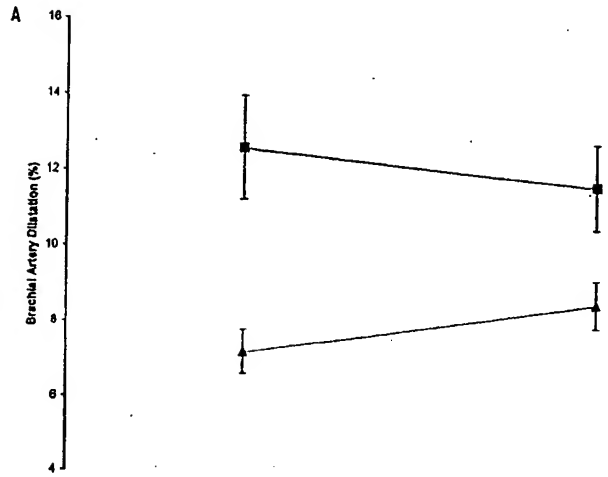
### Endothelial-Independent Dilatation: GTN

There was no significant change in response to GTN on either folic acid (12.59±6.5% to 11.58±5.39%, $P$=0.28, and 0.374±0.136 cm to 0.35±0.129 cm, $P$=0.4) or placebo (12.93±5.71% to 13.75±6.46%, $P$=0.32, and 0.390±0.119 cm to 0.404±0.170 cm, $P$=0.5). There was no significant change in resting heart rate or supine blood pressure after folic acid or placebo.

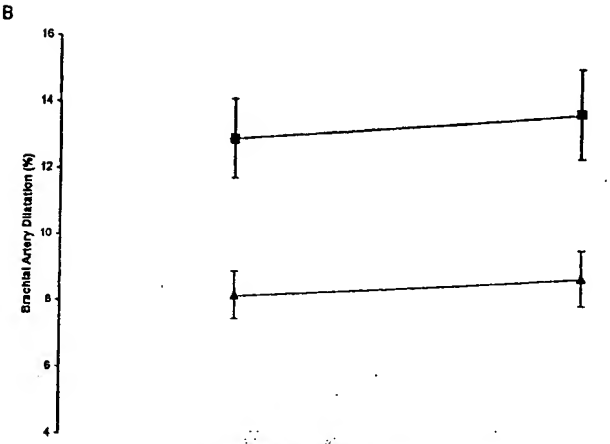
## Discussion

This study shows that in children with CRF, supplementation with high-dose folic acid for 8 weeks results in reduction in homocysteine levels, decrease in LDL susceptibility to oxidation, and improvement in endothelial function. These encouraging findings contrast with the disappointing effects of folic acid supplementation on vascular function in adults with renal disease.[1]

Increased cardiovascular mortality and morbidity is well recognized among adults with CRF.[21] The adverse impact of CRF on cardiovascular mortality and morbidity in the young is, however, even greater, with a 500× higher rate of cardiovascular death than a control population.[22] Homocysteine levels are consistently elevated in adults with CRF, and this has been suggested to play a role in the pathogenesis of atherosclerosis, especially in view of its strong association with death from vascular disease in the non-uremic population.[4,6,23] A high prevalence of other risk factors exists in CRF, but an independent association has been found between elevated total homocysteine levels and the risk of myocardial infarction.[24] The data on homocysteine in children is limited. Elevated total homocysteine levels (12.6±5.2 vs 8.2±3.3 μmol/L, $P$=0.004) have been reported in CRF children compared with controls.[25] Total homocysteine levels at entry to our study (9.85±3.57 μmol/L) were also elevated in comparison to these controls.




(■ = GTN p=ns, ▲=FMD p= 0.036 ).




(■ = GTN p=ns, ▲=FMD p=ns).

Change in mean (±SEM) brachial artery dilatation on folic acid (A) and placebo (B).

Homocysteine levels can be lowered with folic acid. This increases tissue methylation of homocysteine to form methionine in both uremic and non-uremic individuals, even in the presence of normal folate levels. Studies in adults with hyperhomocystinemia and hypercholesterolemia have shown improvement in endothelial function as a consequence of lowering total homocysteine with folic acid.[11,13,26] Similar studies in CRF have been disappointing. In patients with CRF and those on dialysis, no improvement in endothelial function has been demonstrated despite significant reductions in homocysteine. Thambyrajah et al[15] recently published a prospective double-blind trial in which 100 adults with a mean glomerular filtration rate of 30 mL/min and a baseline total homocysteine of 20.1 μmol/L were randomized to either folic acid or placebo. They achieved mean serum folate levels of 39 μg/L and red cell folate levels of 739 μmol/L with 5 mg of folic acid for 12 weeks. These values were lower than those achieved in this study. No improvement in endothelial function (using FMD) was seen despite a significant reduction in total homocysteine. Van Guldener et al[27] treated 30 adults on peritoneal dialysis for 12 weeks with 5 mg of folic acid alone or together with 4 g of betaine (an additional co-factor) followed by 1 or 5 mg of folic acid for 40 weeks. Total homocysteine levels were grossly elevated (42.6 μmol/L) at the beginning of the study and normalized in 40% of patients without any improvement in FMD. In a further attempt to demonstrate long-term clinical benefit from folic acid administration, no improvement in endothelial function was seen after 52 weeks in adult hemodialysis patients, despite a significant reduction in homocysteine levels.[17] Similarly in another population of adults on hemodialysis, carotid artery distensibility and compliance did not change after folic acid supplementation.[27] The explanation for these largely negative studies may be due to the particularly aggressive complex nature of the vascular disease, the inability to normalize homocysteine levels in CRF,[14,28] abnormal folate metabolism, or inadequate folate supplementation.[29]

We chose to evaluate children because this allowed us to study the process of atherosclerosis early in its natural history, when it is potentially more responsive to intervention. In addition, the young population provided an opportunity to minimize the unquantifiable impact of lifelong confounding risk factors on endothelial function. We excluded children with CRF secondary to inflammatory diseases, diabetes, and hypertension because these are known to have a major impact on vascular function, even in the absence of renal impairment.[30] We did not preselect our study population on the basis of FMD or clinical severity of disease so that they would be representative of the effect of CRF in young subjects.

The technique of FMD developed by our group is ideally suited to this study. It is noninvasive, reproducible, and well validated as a measure of NO-dependent vasodilatation and, hence, endothelial function in conduit arteries.[31] There is good correlation between endothelial-dependent responses in the coronary and forearm circulations.[32] The impact of a range of interventions on FMD is well reported both by our group and others in both children and adults with cardiovascular risk factors.

The dose of folic acid in our study produced serum and red cell folate levels higher than in most published clinical intervention studies on CRF patients in the literature, in which endothelial function was the primary endpoint. Variations between 1 mg and 60 mg daily have been used in the renal adult literature with no extra benefit on homocysteine levels conferred by the higher doses. Duration of treatment in adult studies varied from 4 weeks to 52 weeks with the maximum effect on homocysteine seen in the first 2 weeks, and no further lowering occurred despite increasing doses of folic acid.[28]

At the end of the folic acid treatment period, homocysteine levels had fallen significantly. There was an 8-week washout period between the treatment phases. Analysis of serum and red cell folic acid levels showed that the subjects who received placebo after the active phase had a reduction in red cell folate levels. This implies that there was a "carry over" from the active phase and that ideally the washout period

**TABLE 3. Vascular Responses to Folic Acid and Placebo**

| | Baseline | After Placebo | | Baseline | After Folic Acid | |
|---|---|---|---|---|---|---|
| FMD, % | 8.2±3.42 | 8.80±4.01 | NS | 7.21±2.8 | 8.49±3.02 | 0.036 |
| FMD, cm | 0.244±0.102 | 0.276±0.104 | NS | 0.217±0.106 | 0.252±0.081 | 0.047 |
| GTN, % | 12.93±5.71 | 13.75±6.46 | NS | 12.59±6.53 | 11.58±5.39 | NS |
| GTN, cm | 0.390±0.119 | 0.404±0.170 | NS | 0.374±0.136 | 0.350±0.129 | NS |
| Arterial diameter, mm | 3.11±0.57 | 3.18±0.59 | NS | 3.13±0.56 | 3.13±0.58 | NS |
| Resting blood flow (VTI), m | 0.058±0.03 | 0.072±0.03 | NS | 0.065±0.04 | 0.074±0.04 | NS |
| Peak reactive hyperaemia, % | 680±540 | 464±233 | NS | 488±221 | 494±232 | NS |

Results are given as mean±SD. NS indicates not significant; VTI, velocity time integral.

could have been longer. There was, however, no carry over effect on homocysteine levels.

There was a significant improvement in FMD during the folic acid treatment phase without change in response to GTN, which suggests a beneficial effect of folic acid on endothelial function after 8 weeks of treatment. It should, however, be noted that the final FMD after placebo and active phases were not significantly different. Our findings must, therefore, be interpreted with caution, and a longer-term trial may be warranted.

The mechanism by which homocysteine exerts its toxic affect on the endothelium is thought principally to be due to the generation of free radical species.[9] In experimental hyperhomocystinemia induced by methionine infusion in volunteers, vitamin C improved endothelial function.[33] In our study, we noted a significant reduction in total homocysteine levels with folic acid in parallel with an increase in LDL resistance to oxidation through measurement of lag times. Total antioxidant activity was also measured, but no significant change was noted; thus, increasing the resistance of LDL oxidation might play an important role in the improvement in endothelial function because oxidized LDL is a potent vascular toxin. Alternatively folate may improve endothelial function via endogenous regeneration of tetrahydrobiopterin,[34] an essential co-factor in NO production, or through a direct antioxidant effect as shown in vitro.[34,35]

The improved ability to support renal function in CRF has increased the importance of prevention and treatment of vascular disease. Children are surviving into adult life with prolonged exposure to uremia, and there is good evidence that vascular disease associated with CRF is aggressive and starts very early. Folic acid is safe, lowers homocysteine, reduces LDL susceptibility to oxidation, and may improve endothelial biology relevant to the development of atherosclerosis. Long-term benefits require further study.

## Acknowledgments

Dr Katy Bennett-Richards was supported by a grant from the British Heart Foundation and National Kidney Research Fund. Mia Kattenhorn was supported by the British Heart Foundation, and Ann Donald was funded by CORDA (Coronary Artery Disease Research Association). Research at the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust benefits from R&D funding received from the NHS executive.

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# High blood triglycerides identified as independent risk factor for stroke

by Laurie LaRusso, MS, ELS

*Most health experts say that high levels of triglycerides and LDL cholesterol are risk factors for heart disease. But the risk may not end there. A recent study published in Circulation: Journal of the American Heart Association is the first to identify high blood triglyceride levels as a strong independent risk factor for stroke.*

## About the study

Researchers from the Bezafibrate Infarction Prevention (BIP) Study Group in Israel followed 11,177 patients between the ages of 40 and 74 who had coronary heart disease (CHD) but no history of stroke or transient ischemic attack (TIA)—"mini-strokes" that last a few minutes and resolve completely within 24 hours. At the beginning of the study, the patients underwent a complete physical exam that included blood tests for cholesterol and triglycerides. In addition, information about medical history, medication use and risk factors for stroke were obtained.

After six to eight years of follow-up, researchers collected the medical records and/or death certificates of these patients. They looked at how many of the patients had strokes or TIAs. They then compared these results to the patients' blood triglyceride levels to determine whether people with higher blood triglyceride levels had more strokes or TIAs than people with lower levels. Because there are so many related risk factors for CHD and stroke, rigorous data analysis was needed to isolate the effect of high triglycerides from the effects of other risk factors such as total cholesterol and LDL cholesterol.

## The findings

Of the 11,177 patients, 941 developed a cerebrovascular disease (CVD), of whom 487 had a stroke or TIA. Analysis of the data showed that patients with triglyceride levels of 200 mg/dL or higher were nearly 30% more likely to have an ischemic stroke or TIA than those with triglyceride levels below 200. An additional finding was that people with lower HDL cholesterol were more likely to have an ischemic stroke or TIA than people with higher HDL levels. This suggests that higher HDL levels would be protective against stroke, much as they seem to protect against CHD.

In order to isolate the effect of high triglycerides on stroke or TIA, researchers had to tease out a number of other risk factors. These included high total cholesterol, high LDL cholesterol, low HDL cholesterol, increasing age, diabetes mellitus, smoking, hypertension, prior heart attack, and being male. Even after accounting for these factors, the results still suggested that high triglycerides are a risk factor for stroke and TIA independent of these other risk factors.

There are several limitations in the design of this study that may have affected the results.

First, the researchers were looking at triglyceride measurements that had been taken six to eight years in the past. It's possible that during the six to eight years between the time of the lipid tests and the time the medical records and death certificates were analyzed, some patients' triglyceride levels changed. In addition, the people in this study all had CHD, a condition that tends to occur at a younger age than stroke and shares many of the same risk factors. If this factor affected the results, it may well have caused the study to underestimate the strength of high triglycerides as a risk factor.

## How does this affect you?

We know from previous research that high levels of LDL cholesterol and triglycerides are risk factors for heart disease. And we know that low levels of HDL cholesterol are also a risk factor for heart disease. The results of

this study suggest that the same is true for stroke and TIA.

Based on these results, you have yet another reason to work on keeping your LDL cholesterol and triglycerides down and your HDL cholesterol levels up. General recommendations for lowering LDL and triglycerides include:

- A diet that contains less than 30% of calories from fat and less than 10% of calories from saturated fat
- Drinking alcoholic beverages only in moderation
- Living an active lifestyle
- Maintaining a healthy weight
- Lipid-lowering medications, if prescribed by your physician

Because these risk factors can be modified by diet and lifestyle, it is important to see your health care provider regularly for check-ups. He or she can monitor your cholesterol and triglyceride levels and suggest ways to lower your risk of stroke.

**SOURCE:**

Tanne D, et al. Blood lipids and first-ever ischemic stroke/transient ischemic attack in the Bezafibrate Infarction Prevention (BIP) Registry: High triglycerides constitute an independent risk factor.
*Circulation.* Dec 11, 2001;104: 2892-2897.

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Last reviewed Dec 17, 2001 by Richard Glickman-Simon, MD

**EXHIBIT F**



**Note:** Performing your original search, ***HDL independent risk factor***, in PubMed will retrieve *1283* citations.



☐ **1:** Eur Heart J. 1998 Oct;19 Suppl M:M8-14. 

## The emergence of triglycerides as a significant independent risk factor in coronary artery disease.

**Assmann G**, **Schulte H**, **Funke H**, **von Eckardstein A**.

Institute of Arteriosclerosis Research, University of Munster, Germany.

The Prospective Cardiovascular Munster (PROCAM) study involved 4849 middle-aged men who were followed up for 8 years to record the incidence of coronary heart disease (CHD) events according to the risk factors present at study entry. The study showed that fasting levels of triglycerides were an independent risk factor for CHD events, irrespective of serum levels of high density lipoprotein cholesterol (HDL-C) or low density lipoprotein cholesterol (LDL-C). Other independent predictors of CHD included serum levels of LDL-C and HDL-C, age, systolic blood pressure, cigarette smoking, diabetes mellitus, a family history of myocardial infarction and angina pectoris, but did not include total serum cholesterol levels. Individuals with an LDL-C/HDL-C ratio > 5 had a 19.2% chance of experiencing a CHD event in the next 8 years. Furthermore, if an LDL-C/HDL-C ratio > 5 was combined with hypertriglyceridaemia (> or = 2.3 mmol. l-1), the risk of CHD increased to 26.9%. The association between hypertriglyceridaemia and CHD events may be related to the presence of atherogenic, triglyceride-rich particles in plasma, such as LDL and very low density lipoproteins. High triglyceride levels may also predispose to thrombosis. Individuals with potentially atherogenic lipid profiles should be managed initially through the introduction of lifestyle changes. However, if these fail to achieve recommended target values, lipid-lowering therapy should be considered.

PMID: 9821011 [PubMed - indexed for MEDLINE]

1: Am J Cardiol. 1991 Jul 15;68(2):187-92. Related Articles, Links

# Frequency of low serum high-density lipoprotein cholesterol levels in hospitalized patients with "desirable" total cholesterol levels.

Ginsburg GS, Safran C, Pasternak RC.

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Because the National Cholesterol Education Program guidelines suggest that levels of total serum cholesterol less than 5.17 mmol/liter (200 mg/dl) are "desirable," we performed a retrospective observational analysis to determine the prevalence of coronary artery disease (CAD) in patients with total cholesterol less than 5.17 mmol/liter (200 mg/dl) and the prevalence of total cholesterol less than 5.17 mmol/liter (200 mg/dl) in patients with CAD by angiography. Cholesterol levels less than 5.17 mmol/liter (200 mg/dl) were found in 1,084 of 2,535 patients (42%) having cholesterol measured on hospital admission; 690 of these 1,084 (64%) had CAD. These patients were mostly men, had a family history of premature CAD, and 60% (414 of 690) had high-density lipoprotein (HDL) cholesterol less than 0.90 mmol/liter (35 mg/dl). In a separate group of patients defined from the same admission population but having angiographically established CAD, 32% (424 of 1,197) had a total cholesterol less than 5.17 mmol/liter (200 mg/dl), 59% of whom (252 of 424) had HDL less than 0.90 mmol/liter (35 mg/dl). An analysis of persons admitted electively for angiography (to exclude any effects of hospitalization per se on serum lipids) revealed a similar proportion of persons with total cholesterol less than 5.17 mmol/liter (200 mg/dl) (35%), CAD (82%), and HDL less than 0.90 mmol/liter (35 mg/dl). (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 2063780 [PubMed - indexed for MEDLINE]

Nov 15 2005 04:49:13

# Diabetes Information

About ► People ▼ Types & Causes ▼ Insulins ► Medications ► Complications

## Types of Diabetes

What Type You Have ▼ Type 1 ► Type 1.5 ► Type 2 ► Pre-Diabetes ► Syndrome X
Polycystic Ovary Disease ► Gestational Diabetes ► Hemocromatosis ► Cystic Fibrosis

### What Type Of Diabetes Do I Have?

### Really Know Your Diabetes Type? You May Be Surprised

**by Ruth Roberts, M.A.**

When you were diagnosed, you were probably told you had either Type 1 or Type 2 diabetes. Clear-cut and tidy. Since diabetes occurs in two types, you have to fit into one of them. Many people do fit clearly into one of these categories, but some do not. Those who clearly fit a typ diagnosis may find the clear lines begin to smudge over time. Are there really only two types Are you really the type you were told you were? Could you even have more than one type of diabetes, and is your original diagnosis still correct after all these years?

Misdiagnosis or an unclear diagnosis of diabetes can create problems in treatment. Misunderstanding the causes and changes in the disease as you age also can lead to mistreatment. For these reasons, a clear understanding of the types of diabetes is essential.

### A Short History Of Types

Described and treated since ancient times, diabetes has certain characteristics that have long been recognized. Before the discovery of insulin, people found to have sugar in their urine under the age of 20 usually died in their youth, while those diagnosed when over the age of 40 could live for many years with this condition.

Beginning in the mid 1920s, those who got diabetes when young (juvenile onset) were put on insulin, and those who got it when older (adult

**Differences In The Three Major Types Of Diabetes**

| | Type 1 | Type 1.5 / LADA | Type 2 |
|---|---|---|---|
| Avg. age at start | 12 | 35 | 60 |
| Typical age at start | 3-40* | 20-70* | 35-80* |
| % of all diabetes | 10% (25%**) | 15% | 75% |
| Insulin problem | absence | deficiency | resistance |
| Antibodies | ICA, IA2, GAD65, IAA | mostly GAD65 | none |
| Early treatment | insulin is vital, diet & exercise changes helpful | pills or insulin vital, diet & exercise changes helpful | pills helpful, die increased activi essential |
| Late treatment | insulin, diet, exercise (occasionally pills) | insulin, pills, diet, exercise | insulin, pills, die exercise |

* may occur at any age
** if all antibody positive cases are included, ie Type1 and Type 1.5

onset) often were not. However, the mechanisms that led to this difference in treatment were unknown. The only marker that differentiated the two types at that time was presence in the urine of moderate or large levels of ketones when blood sugars were high. If significant ketones were present, the person could not make enough insulin, needed injected insulin to control the blood sugar, and was called insulin-dependent.



In the early 1980s a breakthrough was made in understanding childhood onset diabetes. It became clear that this early onset form was actually an autoimmune disease in which the bo destroyed its own beta cells. The antibodies that the immune system put out during this atta distinguished it from adult onset diabetes. For the first time, one type of diabetes had a clear cause that made it different.

Definitions became clearer. Type 1, called IDDM (insulin-dependent diabetes mellitus), now w recognized as an autoimmune disease that appeared primarily in childhood or adolescence. N the final phases of the attack, the person stops producing insulin and requires injected insulir the time of diagnosis, such a person often has excessive thirst and urination, has lost a lot of weight, and has an extremely high blood sugar. This person is normal weight or thin when Ty diabetes starts and may stay relatively trim through life. Type 1 occurs in about 10% of all people who have diabetes. Treatment for this type revolves around adjusting the dosages an number of insulin injections to match diet and exercise.

Type 2 or NIDDM or non-insulin-dependent diabetes mellitus, on the other hand, was describe high blood sugars occurring in a person over 40 who is overweight and sedentary and also ha family history of this type of diabetes. At the time of diagnosis, there may be no symptoms, the person may have mild symptoms, such as blurred vision or more than normal thirst and urination. The person continues to make insulin, but the insulin production is not sufficient to keep blood sugars normal. Treatment for Type 2 diabetes revolves around varied combinatio diet, exercise, medications, and/or insulin injections.

Note that the use of insulin does not make someone "insulin-dependent" or a Type 1! Some 3 40% of those with Type 2 use insulin, but even when insulin is used, this type of diabetes continues to be non-insulin dependent diabetes mellitus or NIDDM, because death will not oc if insulin is discontinued. Some 90% of people with diabetes are considered to have Type 2.

In the early 1990s the definition of Type 2 was further refined to distinguish those with and without Syndrome X. Syndrome X is strongly associated with insulin resistance and with high total cholesterol (over 200), high triglycerides (also over 200), low HDL (under 40 mg/dl), hi blood pressure, and gout.

Those with an apple figure, who carry excess weight predominantly in their abdomen, are at highest risk of developing Syndrome X. The cholesterol and blood pressure problems associat with Syndrome X trigger accelerated cardiovascular disease, which can lead to heart attack, stroke, and kidney disease.

Syndrome X includes all those people who have resistance to insulin. Some 25% of American into this high risk category, although only about 30% of them will develop Type 2 diabetes at some time in their lives. Type 2 diabetes occurs when the body can no longer produce enoug insulin to keep up with the increased need for insulin. People with Syndrome X also tend to develop high blood pressure because of this insulin resistance.

Not all of those typically classified as Type 2 have insulin resistance and Syndrome X, howev As evidence of this, a study of people with Type 2 was done in Bruneck, Italy, and published Diabetes in October, 1998. Eighty-four percent of the people in the study had insulin resistan while 16% did not. Are these 16% nonetheless to be called Type 2?

When "Type 2" occurs without insulin resistance, it may be referred to as Type 1.5 or Type 2 (for insulin sensitive) or Type 2-d (for insulin deficient). Type 1.5 occurs in adults who usuall lean or normal weight. These people have normal insulin sensitivity but, like other people wit

Type 1, their insulin production is deficient. When their blood sugars are controlled, they usu do not have the high risk for cholesterol, blood pressure, or cardiac and vascular problems typically found in true Type 2 diabetes. This type of diabetes shares characteristics of both Ty and Type 2. Of all the people with diabetes, roughly 10% will have classic Type 1, 75% will h Type 2 (insulin resistant), and another 15% will have Type 1.5.

In their book, **Diabetes, Type 2 and What To Do** (revised October, 1998), Virginia Valentir June Biermann and Barbara Toohey relate that in their 1993 edition of the book, they describ June who developed diabetes in her sixties as a lean Type 2-d. She was similar to the many people in the 16% group in the Italian study described earlier. In 1998, they defined June as Type 1 who got diabetes later in life. They feel this description more closely follows the Amer Diabetes Association revised system, as published in Diabetes Care, January 1998, in which T 1's are insulin deficient and Type 2s are basically insulin resistant. I prefer to keep the third category, Type 1.5, which clearly defines a group that represents a sizable portion (about 16 of those who have diabetes but are neither ketosis-prone nor insulin-resistant.

Other forms of insulin resistant diabetes also can be seen in gestational diabetes, polycystic ov disease, acanthosis nigricans, and maturity-onset diabetes of the young or MODY. Insulin resistant diabetes can also be unmasked by medications like prednisone. In rare cases, nonresistant forms of diabetes may also be seen following trauma to the pancreas or pancrea surgery. This last form is insulin dependent because no insulin can be produced once the pancreas is removed or severely damaged.

Most people with diabetes have Type 1, Type 1.5 or Type 2. As more is known about the cau of diabetes and more treatments are developed, more types or sub types are certain to be defined.

## Why Is Knowing Your Type Important?

Properly understanding your type of diabetes lets you know whether you have been correctly diagnosed, but more importantly, it makes you aware of whether or not you are receiving co treatment. For example, a person diagnosed with Type 1 diabetes needs insulin right away si destruction of beta cells has been going on for awhile. Not until about 90% of the beta cells a destroyed does someone typically begin to have symptoms. If the person does not clearly fit model for Type 1, a diagnosis of Type 2 may be made and oral agents may be prescribed, ev though little insulin production capability remains.

If they are lucky, these agents might stimulate the few active beta cells to produce more inst for a short time, and the blood sugar may be controlled temporarily. However, soon an oral a will fail, and injected insulin will be needed. If the oral agent does not work, the person will continue to be very sick until insulin is started. If Type 1 had been recognized right away thr an antibody test, using insulin immediately might lead to fewer problems with control, since t often allows insulin production to continue for a longer period of time. Blood sugar control is easier when beta cells continue to work.

Knowing your diabetes type can also give you a better understanding of the changes that ma occur to you as you age and your disease progresses. For example, if you have had insulin-resistant diabetes for several years and it has become harder to control on a sulfonylurea medication, you may find that your C-peptide level is now low, and insulin may now be requi If your C-peptide is normal, adding another oral agent and paying closer attention to your fo and exercise choices may be all that is needed. Both situations can occur as the disease progresses and are not necessarily a result of poor practices on your part.

Dr. David Bell, a clinician and researcher in Birmingham, Alabama, wanted to see if he could a group of people with Type 2 diabetes who were already on insulin and eliminate insulin use substituting a combination of oral medications. He first tested C-peptide levels and chose onl those who had normal levels. Of the 130 people with adequate C-peptide levels in his 1997

study, 100 were able to discontinue insulin use altogether and control their diabetes on vario doses of glyburide and metformin, medications that were not available when many of the patient's insulin use was begun. Dr. Bell found that their overall control, measured by a HbA1 level, was better on these two oral medications than it had been on two doses of insulin a da Other people in the study were able to improve their hemoglobin levels by using glyburide, metformin, and one dose of insulin at dinner or nighttime.

Researchers have determined that the Type 2 patients who are most likely to control their bl sugars on a combination of oral agents alone are those least overweight (BMI of 30 or less), shortest duration of insulin use, and C-peptide levels normal or only slightly low.

## Who Is Most Likely To Be Misdiagnosed?

Many people with Type 2 diabetes are not diagnosed at all. This rampant problem means son million Americans do not know they have this disease. Symptoms are usually minimal or nonexistent, sometimes for years, and so the person is simply not treated for diabetes. An elevated blood sugar is only picked up when the person goes in for a routine physical exam o visits the doctor for another problem, like a cold or a flu.

Among people who are diagnosed with diabetes, misdiagnosis of the type happens most ofte when the person does not have the body type or age expected for Type 1 or Type 2. For example, a person who is 38 and slender has mildly elevated blood sugars. Is this person Ty or Type 2? He is older and his blood sugar may not be as high as a typical Type 1, but he is t thin for a true Type 2. Perhaps he has Type 1.5 with diminished insulin production but no ins resistance. If the older person who is slim has very high blood sugars when diagnosed, the t more likely will be thought to be Type 1.

Or consider a child of 14 who is 40 pounds overweight and has high blood sugars. Does this c have Type 1, Type 2, or MODY (a different type of diabetes genetically predetermined)? Due overeating, poor nutrition habits and a sedentary lifestyle, more and more children are now developing Type 2 at an early age. In fact, Dr. Gerald Bernstein, president of the American Diabetes Association, says one-fourth of new cases in people under age 20 are now Type 2. I the Journal of the AMA, November, 1998, researchers are recommending that diabetes screening be considered for sedentary, overweight people as young as 15 as a way to preven the complications that years of high blood sugars can cause.

What about the person who is 50 years old, has high blood sugars, is 15 pounds overweight, has a pear shaped body? Is she Type 1 or Type 2? She could be an older-than-usual Type 1 c she could be a Type 2 with a strong family background of diabetes, meaning that a modest weight gain is all that was needed for diabetes. This is especially true if body fat is high and deposited intraperitoneally (in the gut).

These cases indicate that people often do not fit into clear profiles. When the traditional profi does not match the person, understanding what may have caused the diabetes and determin how it should be treated is often problematic.

## Does Your Type Ever Change?

Blurring of the lines between Type 1 and Type 2 diabetes is becoming increasingly common. I to aging or the general progress of the disease, people with one type of diabetes tend to take characteristics of the other. As a result, some people with diabetes may have characteristics both types.

If Type 1's begin to exercise less and gain weight around the middle, as many people do whe

needed to properly diagnose and treat diabetes.

In summary, our understanding of diabetes and the lab tests useful to us continues to evolve understand your situation as information changes, you want to ask specific questions about y diagnosis and treatment. An informed, questioning approach will increase your likelihood of receiving the best care.

## Mis-Typing Is Common

When you were diagnosed, you were probably told you had either Type 1 or Type 2 diabetes: clear-cut and tidy. Since diabetes occurs in two types, you have to fit into one of them, or so used to be thought. Many people do fit clearly into one of these categories but not everyone. Even those who clearly fit one type at diagnosis may find the lines begin to smudge over time Are there really only two types? Are you really the type you were told you were? Could you h more than one type of diabetes? And is your original diagnosis still correct after several years

Misdiagnosis or an unclear diagnosis of diabetes can lead to problems in treatment and healtl Misunderstanding changes in the disease as you age can also lead to mistreatment. The lack way to clearly define the different types of diabetes has allowed people to be misdiagnosed, especially if clarification is based on the typical body type or age. Today we have better lab te to differentiate Type 1 and Type 2, but they often are not done and even when they are, the diagnosis may not be definitive.

When a person does not match a typical profile, mistakes can be made in creating a treatmer plan. People who have Type 1 diabetes must have injected insulin to live because they produ little or no insulin themselves. People who have Type 2 will need oral medications or insulin, depending on their lifestyle and the severity of their disease. Although they make take insulir good control, they are not insulin dependent as is the person with Type 1.

In fact most people who use insulin are not actually insulin dependent. The number of people with Type 2 diabetes who use insulin is two or three times as large as those with true insulin dependence or Type 1. Some 30 to 40% of people with Type 2 diabetes require insulin to maintain control, but even when insulin is used, this type of diabetes continues to be non-ins dependent diabetes mellitus or NIDDM, because death will not occur over a few days if insuli discontinued.



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Many thanks to Helen Oswalt, editor of the Scripps Whittier *Keeping In Touch Newsletter*, for many helpful editing suggestions.

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they age, they may become not only insulin deficient but also insulin resistant. They then ca develop the cardiac risks associated with Syndrome X and require medications to lower cholesterol and blood pressure. They will require more insulin to control their blood sugars, a certain medications typically used in Type 2 diabetes, such as Glucophage or Rezulin, may he their control.

On the other hand, as Type 2 diabetes progresses, especially if it is not well-controlled and th pancreas is placed under additional stress, insulin production may diminish to a point where i can no longer keep up with need. A sulfonylurea may no longer be able to stimulate the beta to produce enough insulin. Medications in addition to sulfonylurea, such as Rezulin, Precose o Prandin, may be needed. As insulin production falls further, injected insulin will be required t keep blood sugars from rising. Some people with Type 2 eventually become totally dependen insulin and can go into ketoacidosis if insulin injections are stopped.

## How Can You Know Your Type At Any Age Or Stage?

When a person does not fit into a clear profile, a diagnosis of Type 1, Type 1.5, or Type 2 is r obvious. A variety of lab tests and clinical signs help to provide the critical information neede correctly determine which type of diabetes the person has.

* **Ketones:** Ketones are a byproduct produced when the body uses large amounts of fat as fi This occurs when carbohydrate is no longer available as fuel due to a lack of insulin. When a urine or blood test shows large amounts of ketones, that person definitely has Type 1 or insu dependent diabetes. (One rare exception is young, black males who can have ketones at diagnosis but regain insulin production.) If insulin is injected before the ketone test is administered, the opportunity to find large amounts of ketones may have passed. The urine c easily be tested for ketones at home with Ketostix or Ketodiastix anytime the blood sugar lev are high.

* **Antibodies:** Type 1 diabetes is an autoimmune disease, so 80 to 90% of the time when T 1 exists, the person is producing antibodies characteristic of Type 1, such as the islet cell antibodies and GAD 64 antibodies. The blood can be tested to see if any of these antibodies a present. If antibodies specific to Type 1 are detected, the person already has or is likely to develop Type 1 diabetes. These tests are currently used in the DPT-1 trial to test relatives of those with Type 1 diabetes and detect who will develop this disease.

* **High triglyceride and low HDL:** Cholesterol problems characterized by high triglyceride low HDL are typical of insulin resistance. These markers for Syndrome X are commonly found Type 2 diabetes. A detailed cholesterol test or lipid profile test will determine this.

* **Uric Acid:** The high uric acid level often found in people with gout is a component of Syndrome X. If a person has a high uric acid level and high blood sugars, he usually has insu resistant, Type 2 diabetes.

* **C-peptide:** If other tests fail to indicate the type of diabetes, a C-peptide test can reveal h much insulin the person is producing. C-peptide is half of the precursor molecule to insulin th split off when insulin is produced by the body. If C-peptide is normal or high, Type 2 diabetes likely. If the level is significantly low, Type 1 diabetes is likely. If the level is near normal but the results are inconclusive. This person may have early Type 1, Type 1.5, or long-term Type When external insulin is controlling the blood sugar, the C-peptide may read low due to suppression of insulin production by the beta cells. This test should be done after insulin has reduced or discontinued, and the blood sugar has risen to 200 mg/dl or over.

When should these tests be used, since lab tests increase health care costs, and no one want unnecessary tests? Use them when a person who is not a clear type is diagnosed with diabet when treatment is not working for unclear reasons. Although these tests often do not tell everything needed for a complete understanding, they can provide more of the clarification needed to properly diagnose and treat diabetes.

EXHIBIT I

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Articles

# Drug-related dyslipidemia after renal transplantation

**AS Mathis, N Dave, GT Knipp, and GS Friedman**

PURPOSE: The frequency, onset, mechanisms, and causes of dyslipidemia after renal transplantation are reviewed in the context of the adverse impact of lipid alterations, recent guidelines, and the available treatment options. SUMMARY: At least 60% of adult renal transplant recipients develop dyslipidemia, which occurs within one month of the initiation of immunosuppressive therapy and continues indefinitely unless treated. Cyclosporine, sirolimus, and prednisone are mainly implicated, and the lipid profile differs between individual agents. In recognition that lipid alterations in these patients are linked with development of ischemic heart disease, vascular mortality, and graft deterioration, the National Kidney Foundation has recently released guidelines suggesting a low-density-lipoprotein (LDL) cholesterol goal of < 100 mg/dL for these patients. Statins and diet therapy are recommended as first-line agents for achieving goal LDL cholesterol levels in this population. Recent evidence proved a reduction in adverse cardiovascular events when fluvastatin was utilized in one large-scale trial. Care should be taken with aggressive dosage adjustment because of the potential for a pharmacokinetic interaction with cyclosporine and a resultant increase in the risk of myopathy or rhabdomyolysis. Other options for improving the lipid profile include modifications in the immunosuppressive regimen, the addition of other lipid-modifying agents, and using alternative lipid-modifying agents. CONCLUSION: Statins and diet therapy should be used as first-line treatments in renal transplant recipients with dyslipidemia. Other strategies, including modification of the immunosuppressive regimen, and the addition of other lipid-modifying agents, have also yielded positive results.